POPULATION ECOLOGY AND REPRODUCTIVE BIOLOGY OF THE ENDANGERED *BUXUS VAHLII* BAILLON (BUXACEAE)

by

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ABSTRACT

Buxus vahlii Baillon is an endangered plant species endemic to Puerto Rico and St. Croix (USVI). There are six known populations, four in Puerto Rico (Rincón, Isabela, two in Bayamón) and two in St. Croix (Frederiksted and Christiansted). The status of these populations was studied to provide information on the ecology of the species. The species showed the ability to adapt to different environmental conditions. Such adaptations include shrubby growth in dry areas where it grows as part of the understory (Rincón, Frederiksted and Christiansted) vs. an arborescent growth form in high precipitation areas (Isabela and Bayamón). Identified human impacts on the studied populations were: development (Frederiksted), illegal immigrants and fires (Rincón), and rock climbing and introduced species (Bayamón). The Isabela and Christiansted populations were not impacted due to their low accessibility. Actualization of the management plan is suggested to reverse the decline of this species and restore the populations.

RESUMEN

Buxus vahlii es una planta en peligro de extinción endémica de Puerto Rico y Santa Cruz (Islas Vírgenes Americanas). Existen seis poblaciones conocidas, cuatro en Puerto Rico (Rincón, Isabela, dos en Bayamón) y dos en Santa Cruz (Frederiksted y Christiansted). Se estudió el estatus de estas poblaciones para proveer información respecto a la ecología de la especie. La especie muestra la habilidad para adaptarse a diferentes condiciones ambientales. Estas adaptaciones incluyen crecimiento arbustivo en áreas secas donde crece como parte del sotobosque (Rincón, Frederiksted y Christiansted) vs. un crecimiento en forma arborescente en áreas de alta precipitación (Isabela y Bayamón). Impactos humanos identificados en las poblaciones estudiadas fueron: desarrollo (Frederiksted), inmigrantes ilegales y fuego (Rincón), y escalamiento de rocas y especies introducidas (Bayamón). Las poblaciones de Isabela y Christiansted no se impactaron debido a su pobre acceso. Se sugiere la actualización del plan de manejo para revertir la declinación de la especie y restaurar las poblaciones.

To the stones that cross my way. They make my feet stronger and my steps steadier. No matter how much painful it can be and how many obstacles would be in my way, I am going to keep walking in the same direction.

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INTRODUCTION

The distribution of the family Buxaceae is nearly cosmopolitan; it includes five genera, of which the largest is *Buxus* L. The members of the family are woody plants that are evergreen or rarely deciduous.

Buxus L. is a widely distributed genus composed of seventy species (Liogier, 1988). The neotropical species of *Buxus* were included in the genus *Buxus* (Urban, 1908-1925) and classified as section *Tricera* according to Mathou 1940 (cited in Köhler, 1981). With about 40 species, section *Tricera* is the largest and most difficult group of the genus, which is also represented by other sections in Africa as well as in Asia and Europe. The distribution of section *Tricera* comprises the Antillean Islands, extending to Central America and Mexico with four species, and reaching South America with one species, which has been recently recorded as occurring in Venezuela. There is a marked center of speciation in Cuba, where more than 30 mostly endemic, often very locally distributed and ecologically specialized species occur. Four or five species occur in Jamaica, three in Puerto Rico, and one each in Martinique and the Bahamas (Köhler, 1981). One species has been reported for Hispaniola (Liogier, 1986) and one species for the Cayman Islands (Proctor, 1984).

A complex analysis of the evolutionary patterns within the genus *Buxus* attempts to reconstruct the history and phylogenetic relationships of this group with its origin in the neotropics. The pollen morphology of *Buxus* in the Caribbean region shows relations to the East African species and certain resemblance to the Macronesian ones; this not only demonstrates the remarkable character of the genus, but seems to be of general interest for the knowledge of the natural ancestry and origin of the Caribbean flora (Köhler, 1981).

Liogier (1988) cited the following species for Puerto Rico: *Buxus laevigata* (Sw.) Spreng., *B. portoricensis* Alain and *B. vahlii* Baillon. Nevertheless, the recent publication of Liogier and Martorell (2000) confirms only two *Buxus* species for Puerto Rico: *B. portoricensis* and *B. vahlii*. Little et al. (1974) classified *B. vahlii* as a "rare shrub." The subject of this investigation, *B. vahlii*, has been described as rare at low and medium altitudes in humid forests of karst topography, in Bayamón, Rincón, and Ponce, Puerto Rico, in St. Croix, and doubtfully in Jamaica (Liogier, 1988). Adams (1972) does not confirm its existence in Jamaica and Britton and Wilson (1924) cite it as endemic to Puerto Rico and St. Croix. *Buxus vahlii* was declared an endangered plant species on August 13 of 1985 (Federal Register 50: 32572), under the Endangered Species Act of 1973.

Extensive studies have been carried out using pollen morphology of the genus *Buxus* in the Caribbean. These studies have given us a better understanding of the patterns of variation and evolution among the species of that genus. Köhler (1981) tried to demonstrate possible evolutionary trends in the pollen characters of the genus. He classified all the species in eight groups based on pollen morphology, and according to him *B. vahlii* is related to *B. retusa* Muell. from Argentina and *B. crassifolia* (Britt.) Urb. and *B. brevipes* Urb. from Cuba, mainly because of the characteristically crenate pollen walls.

Buxus vahlii is an evergreen shrub that may reach 15 m in height, with a stem diameter of up to 13 cm. The twigs have two characteristic grooves below each pair of leaves. The leaves are simple, opposite, and oblong or obovate. The species generally flowers and produces seeds between December and April. The flowers are small and unisexual; they are borne together in an axillary cluster on the same plant. The fruit is a capsule of approximately 6 mm in length, with three horns. This characteristic contributed to its common name, “three horned devil” (USFWS, 1987).

In St. Croix, *Buxus vahlii* is found in the Subtropical Dry Forest life zone (Ewel and Whitmore, 1973), in contrast with Puerto Rico, where it is in the drier areas of the Subtropical Moist Forest zone, in the municipalities of Bayamón, Isabela, and Rincón.

In Hato Tejas, Bayamón, *Buxus vahlii* is associated with two subcanopy trees: *Coccoloba diversifolia* Jacq. and *Casearia silvestris* Sw. Commonly, species such as *Pitcairnia angustifolia* Aiton, *Zamia debilis* L.f. and *Anthurium crenatum* (L.) Kunth also occur with it (USFWS, 1987).

In the Guajataca State Forest, Isabela, the majority of the forest has been cut, burned, or cultivated in the past. When the land was acquired by the Reconstruction Administration of Puerto Rico in 1935, most of the area consisted of shrubland or pasture (DNR, 1976). In the past, the sumps were planted with coffee, which has been substituted with plantations of kadam, maria, and mahoe (Vivaldi, 1986). Little and Wadsworth (1964) and Little et al. (1974) reported 186 species of trees in the Guajataca State Forest; of these 47 have been identified as rare or endangered (Vivaldi, 1986). The population of *Buxus vahlii* in this forest is on the east-facing slope of a mogote, 31 m south of the Juan Pérez trail. In this population fifteen adult plants were labeled in an earlier study (Kolterman and Breckon, 1993).

At Punta Higüero, Rincón, the vegetation is characteristic of a coastal forest. *Buxus vahlii* is found associated with species such as *Guettarda scabra* (L.) Vent., *Coccoloba uvifera* (L.) L., *Erithalis fruticosa* L., and some introduced species, such as *Terminalia catappa* L. Observations of *Buxus vahlii* in the Rincón population have provided preliminary information on population structure, phenology, and reproduction.

The floral morphology suggests that pollination is carried out by wind or insects, such as the introduced honeybee (Kolterman and Breckon, 1993), and self-pollination is also considered to be viable. The examination of the plant does not provide evidence for vegetative regeneration, so it is not very probable that this type of regeneration contributes significantly to the growth or maintenance of the population (USFWS, 1987).

The general purpose of this work was to study aspects of the population ecology and reproductive biology of *Buxus vahlii* in order to:

1. Determine the distribution, abundance and ecology of *Buxus vahlii* in Rincón, Isabela and Bayamón populations.
2. Visit other well-known or reported populations for the species in St. Croix.
3. Determine the phenology and reproductive biology of *Buxus vahlii,* emphasizing the flowering periodicity, pollination mechanisms, production and viability of seeds, establishment and growth of seedlings.
4. Evaluate intra- and interpopulation genetic diversity by means of leaf morphometric studies and, if possible, electrophoresis studies.

5. Collaborate with the U.S. Fish and Wildlife Service (USFWS) and the Puerto Rico Department of Natural and Environmental Resources in the improvement, actualization and implementation of the *Buxus vahlii* recovery plan.

LITERATURE REVIEW

There are several reasons for justifying the preservation and recovery of rare and endangered plant species. Among these are their functions within natural ecosystems and their demonstrated or potential uses for humans, such as food, fuel, or medicines.

Economic uses of *Buxus*

Members of this genus have wood of commercial value. The chemical structure of lignins in *B. sempervirens* wood samples shows a progressive gradation from normal wood to reaction wood, and lignins in samples with a pronounced compressed character were found to have structural similarities to those of gymnosperms (Baillères et al., 1997). The use of *Buxus* wood for commercial purposes has not been reported in the Caribbean (INDAF, 1970; Little et al., 1974). Little et al. (1974) describe the wood of *B. vahlii* as yellowish and hard. They propose the use of this species for hedges, because like *Buxus sempervirens* it has attractive foliage, and can also be used as a cultivated ornamental.

Phytochemical studies of several members of the genus *Buxus* have been carried out. A red carotenoid named anhydroeschscholtzxanthine, which has not been isolated from any other living organism, has been isolated from the leaves of *B. sempervirens* (Ida et al., 1995). In *B. sempervirens*, an ornamental species, "watery extracts of this plant have varied uses in the indigenous medicine for the treatment of several ailments" (Atta-ur Rahman et al., 1991a). Some 150 new alkaloids have been isolated from *B. sempervirens, B. papillosa* and *B. hildebrandtii* (Atta-ur Rahman et al., 1991b, 1992, 1993). *Buxus papillosa* has also been used for the treatment of malaria, rheumatism, and dermatologic diseases (Atta-ur Rahman et al., 1994). A recent study showed interesting properties of *B. sempervirens* in the treatment of human acquired immunodeficiency virus according to Durand 1996 (cited in Fourneau et al., 1997).

In spite of the chemical constituents of pharmacological interest reported for some species of *Buxus* and the fact that the majority of the species of the genus are Antillean (Liogier, 1988), there are no reports on the phytochemistry of the genus in the Caribbean species, nor do data on their medicinal uses in Puerto Rico and the Caribbean appear in the literature (Núñez-Meléndez, 1982; Liogier, 1990; Tomas, 1997).

A very odorous compound responsible for the odor of the "Sauvignon grapes," 4-mercapto-4-methylpentan-2-one, has been identified in *B. sempervirens*. The term boxwood has been used for the description of the aroma of Sauvignon vines (Tominaga and Dubourdieu, 1997).

Rare and endangered species

Rarity and endangerment are not synonymous. Rarity is an expression of the pattern of distribution and abundance of a species at a specified time. Endangerment (also known as threat) refers to factors (generally anthropogenic) that may make a species more susceptible to decline or extinction. Various historical, ecological and genetic hypotheses have been presented as to the causes and consequences of rarity (Stebbins, 1980; Baskauf et al., 1994; Morse, 1996; Crins, 1997). Since it has become clear that no single explanation can be applied to all cases, field studies are required for the identification of these factors (Henifin et al., 1981).

Few endangered plant species have ecological studies in sufficient detail (Falk and Olwell, 1992). *Buxus vahlii* was the object of preliminary studies under cooperative agreements between the U.S. Department of the Interior, Fish and Wildlife Service and the Department of Biology of the University of Puerto Rico, Mayagüez Campus. These studies were carried out between the years 1991 and 1993 (Kolterman and Breckon, 1993).

Buxus vahlii is one of the endangered plant species of Puerto Rico that has been cultivated in the Fairchild Botanical Garden in Florida, with the purpose of preserving the species *ex situ* and eventually reintroducing the propagated plants into protected areas and historic localities where the species used to grow.

Threats to rare species

Rare and endangered plant species owe their rarity to different natural or anthropogenic factors or to combinations of them (Howard, 1977; Reveal, 1981). The frequency of hurricanes has been well documented in the Caribbean islands. Puerto Rico lies directly in the Caribbean hurricane belt, and a hurricane or tropical storm passes over the island or its adjacent coastal waters about once every four years (Bush et al., 1995). The forest destruction may be followed by the regrowth of different rapid growing, shade-producing species and the effects of the different types of cover are difficult to evaluate (Howard, 1977).

Power-Bratton and White (1980) found that the greatest threat to rare plant populations in most preserved sites is anthropogenic interference. Some of the effects of humans on vegetation are the animals they keep, the crops they cultivate, and their activities in mining, forestry, housing and tourism (Howard, 1977). Several human impacts have been reported for *Buxus vahlii* populations. The Rincón population has been affected by occasional fires (USFWS, 1987), tree cuts, and the passage of illegal immigrants through the population (Kolterman and Breckon, 1992). The Bayamón population is located on private land surrounded by urban and industrial development. Even minor expansion of existing facilities would eliminate the remaining plants (USFWS, 1987).

<u>Habitat requirements</u>

Rare plants and their habitats, as well as rare habitats *per se*, comprise integral components of the biodiversity of any geographically defined area (Crins, 1997). Many states with the highest indices of plant diversity, including Hawaii, California, Texas, Florida and Puerto Rico, have major areas of habitat, encompassing whole endemic community types, on land that is unprotected and under active commercial development (Falk and Olwell, 1992). Site selection considerations for rare plant introductions must include physical, biological, logistical and historical criteria. Additionally, the nature of rarity has to be incorporated into site-selection deliberations (Fiedler and Laven, 1996).

Bisse (1988) describes Cuban *Buxus* as typical species of small xerophytic thickets, between 8 and 10 m in elevation, developed over soils originated from ultrabasic rock. In Puerto Rico, *Buxus portoricensis* has been recorded from wet forests at middle elevations, mostly on serpentine soil, and *Buxus vahlii* has been described as rare in moist limestone forest at lower to middle elevations (Liogier and Martorell, 2000).

Abundance and demography

Conservation of genetic diversity in tropical plants, rare as well as common, is challenging and requires knowledge about the distribution and abundance of species (Bawa and Ashton, 1991). Plant survival and fecundity are determined largely by the size and physiological status of the individual plant, which are presumed to be a function of local conditions and resource availability (Holsinger and Gottlieb, 1991). Despite the paucity of data on population size variation in plants, natural history observations indicate that populations of some species are large, continuous and stable; some are regularly small and sparsely distributed, whereas others fluctuate dramatically from season to season (Barrett and Kohn, 1991).

Species occurring in few sites or in small populations will be the most vulnerable (Falk and Olwell, 1992). It is often assumed that rare and endangered species inevitably occur in small populations that are geographically isolated from one another (Barrett and Kohn, 1991). Some of the rare species in the forest may be last survivors of species once more common in earlier successional stages, and some may be accidentals that became established through good fortune outside the habitat to which they are optimally adapted. Potential consequences of a low-density uniform dispersion of adult trees in tropical species might include lower outcrossing success, reduction in deme size, and requirements for long-distance pollination (Hubbell, 1979). Both spatial variation in post-dispersal seed predation and differences in predation between species are important elements that facilitate the coexistence of different plant species. Where microsites are limiting, selective post-dispersal seed predators can influence pre-emptive competition for these microsites (Hulme, 1998).

Reproductive biology

Information on reproduction and life history is important for the conservation of endangered species. A population's reproductive strategy affects its effective population size, the distribution of genetic variation, and the design of sampling and management strategies for conservation (Olfelt et al., 1998).

Phenological studies document the patterns in the vegetative and reproductive status of individuals in a plant population over time. Consequently, studies of flowering phenology have been used to address ecological and evolutionary questions concerning intra- and interspecific competition, community structure, keystone relationships, coevolution, animal foraging behavior, phylogenetic constraints, and continent-wide patterns (Stiles, 1977; Primack, 1980; Augspurger, 1981; Crepet, 1983; Gross and Werner, 1983; Kochmer and Handel, 1986; Skeate, 1987; Pors and Werner, 1989; Hamrick and Murawski, 1990; Dieringer, 1991; Ollerton, 1996; Ollerton and Lack, 1998).

The tropical forests of the New World have been characterized by the great diversity of plant and animal species with high degrees of interaction, showing by consequence complex phenological patterns (Puentes et al., 1993). Newstrom et al. (1994) proposed a new classification for plant phenology based on flowering patterns that included frequency time series and subsidiary classes based on other quantitative descriptors such as regularity, duration, amplitude, date, synchrony and the conceptual framework that separates patterns at each level of analysis. Natural selection should produce a regular sequence of flowering times, in order to minimize competition between plant species for pollinators or to minimize interspecific hybridization (Poole and Rathcke, 1979; Cole, 1981).

Plant breeding systems are under genetic control and can themselves be selected for. They are rarely fixed and static, but are fluid and respond to selection pressures in an infinite variety of subtle and interrelated ways (Richards, 1997). Reich and Borchert (1984) showed that, in general, the timing of leaf fall and bud break and, in many species, anthesis is determined to a large extent by changes in tree water status. These phenomena, in turn, are a function of the interaction between the water status of the environment and the structural and functional state of the tree.

Seed germination and dispersal

Seeds provide the most natural means of plant reproduction, preservation of genetic variability, transportation and propagation of phanerogamic plants (Vázquez and Rojas, 1996). The duration of seeds in nature varies widely among species. Seed germination percentage and rate can be influenced by the altitude at which seeds were collected and by seed size (Vera, 1997). Augspurger (1984) established a positive correlation between the seed survival in shade and in sun; shade tolerance varied widely and continuously among species: it correlated with adult wood density, an indicator of growth rate and successional status, but not with the dry weight of seed reserves. Bergelson and Perry (1989), in a greenhouse experiment, found that the probability of emergence significantly decreased with an increase in total seed density and the rate of emergence accelerated in the presence of previously planted seeds. This acceleration involved a response to leachates from previously germinated seeds.

Seed dispersal mechanisms can have a direct impact on the genetic structure of populations. Species whose seeds are dispersed near the maternal plant (e.g. by gravity or wind dispersal) or species whose seeds are deposited in clumps or patches should have more fine-scale genetic structure than species whose seeds are dispersed by mobile animals (Hamrick et al., 1993).

Germination experiments with *Buxus vahlii* have been carried out in Puerto Rico (Kolterman and Breckon, 1993). The germination of seeds was done using scarification, which proved to be unnecessary; in fact, the controls germinated more readily than the treated seeds. Contamination by fungi was observed on these trials. Greenhouse experiments were also carried out, without any success, perhaps because the seeds had been stored for some time. In St. Croix, Daly and Zimmerman (2000) tried to germinate *B. vahlii* with several treatments: control, 24 h water soak, boiling water or 1 h gibberellic acid soak. *Buxus vahlii* did not respond to any of the treatments.

Population genetics

Measurements of morphological diversity are of interest in ecological and genetic studies. Techniques of description and comparison of shapes of structures are needed in any systematic study (whether phenetic or cladistic) that is based on the morphology of organisms (Rohlf, 1990). In different organisms (plants, shells, snails and butterflies) the first polymorphisms known were visible variants that affected color, shape, pattern or other morphological aspects (Hedrick, 1999).

The most easily obtained assessment of genetic variation is done by measuring morphological or phenotypical variation. Morphology has the advantage of requiring neither breeding nor laboratory studies and, most important, such work can be done directly from field collections. Another distinct advantage of studying morphological variation is that phenotypic characters are often ecologically adaptative. Such morphological variation is often assumed to be indicative of genotypic variation, local differentiation or ecotypes (Holsinger and Gottlieb, 1991).

Morphometrics is the quantitative description, analysis, and interpretation of shape and shape variation in biology. The use of leaf morphology to establish differences among populations has been done with Caribbean cycads (*Zamia*). Leaflet morphology has long been important in the taxonomy of this genus (Newell, 1989). Studies have been carried out using leaves of members of the genus *Buxus* for the Caribbean. Köhler (1984) used leaf venation patterns for the studies of 47 species of *Buxus* and presented taxonomic criteria for the classification and evolution of the studied species. Four venation patterns and their evolutionary trends were described.

METHODOLOGY

Study sites

Herbaria (UPR, SJ, UPRRP, MAPR) and the personal collections of Rudy O'Reilly in St. Croix were consulted in search of new localities and phenological information. In December 1997, the *Buxus* collections at two herbaria in Cuba (HAC, HAJB) were consulted. Consultation with botanists in Puerto Rico and St. Croix were also used in order to try to locate additional populations. When available, information on the ownership of the places where *Buxus vahlii* grows was obtained. This information is very important since the Endangered Species Act does not offer much protection for plants on private property.

Six populations were previously reported for *Buxus vahlii*. Four populations are know from Puerto Rico: one in Rincón (located on land owned by the Commonwealth of Puerto Rico, and managed by the Puerto Rico Electricity Power Authority), one in Isabela (located in a public forest, the Guajataca Forest), and two in Bayamón (one on public property, managed by the Science Park and one on privately owned land); this population is adjacent to a major commercial development area. The other two populations, both on private lands, are in St Croix, USVI: one near Frederiksted on the west coast and one near Christiansted on the north.

The location of the *B. vahlii* populations were determined using the following USGS topographic maps: Rincón quadrangle (Rincón population), Quebradillas quadrangle (Isabela population), Bayamón quadrangle (Bayamón population), Frederiksted quadrangle (Frederiksted population) and Christiansted quadrangle (Christiansted population). In 1996, two preliminary visits were conducted to Rincón and Isabela localities to confirm the existence of both populations. Subsequent visits were made to both populations. In January 1999, the Christiansted and Frederiksted (St. Croix) populations were visited. In October 2000, the Bayamón, Science Park, population was visited. Data on habitat and associated plant species were recorded for all the visited populations. Vouchers of *B. vahlii* in all the studied populations were deposited in the herbarium of the Biology Department of the University of Puerto Rico, Mayagüez Campus (MAPR).

Population ecology

This study of the population ecology of *Buxus vahlii* focused on the populations in Puerto Rico (Rincón, Isabela and Bayamón "Science Park"). In the Rincón population, 630 adult plants with a principal stem of basal diameter ≥ 1.0 cm and ≥ 0.5 m height were tagged (Kolterman and Breckon, 1992, 1993). For each of these plants the number of main stems from the base, the basal diameter [calculated as the square root of the sum of squares of the individual basal diameters, $(\Sigma d_i^2)^{1/2}$, for individuals with more than one main stem], the approximated height and presence of reproductive structures were recorded during March, September, and October of 1992 and March, 1993. During August 1997, 52 randomly selected individuals were measured again in order to compare these data with the previous study and measure possible population changes over this period of time. There were very few previous data from the Isabela population; only 15 individuals were measured by Kolterman and Breckon (1993). During August and September 1997, 168 new randomly selected individuals were measured and their diameter and height were recorded. There were no previous data for the Bayamón population. During October 2000, 21 individuals were measured and their diameter and height were recorded. Seedlings were counted and the limits of the populations were determined for this species at each locality. The size structure of the populations was constructed based on the plants' measurements. During May 1999, the St. Croix populations were visited and field observations were done in both populations. Considering the non-normal distribution (skewed towards small-sized organisms) of the *B. vahlii* populations, a Kruskal-Wallis statistical test was used to analyze these data.

Reproductive biology

Phenology – Phenological studies can help determine possible differences in the foliage, flower, and fruit production between individuals within and among populations. In the Rincón population, one branch from each of several different individuals was selected, using accessibility and plant health as criteria. The number of individuals sampled each month varied between 20 and 54. Phenological observations were conducted for one year, between June 1997 and May 1998, at the Rincón locality. Every month a census was carried out; the production of new leaves was recorded, and flowers and fruits were counted, noting their state of development. The phenological data were compiled by counting the number of plants in each phenophase (new vegetative growth, inflorescences in bud, open flowers, new fruits and mature fruits) observed during one or more visits during the month. For the Isabela and Bayamón populations, the reproductive state of the individuals was recorded during field visits to both populations. For comparative purposes, the results of this section are shown in terms of percentages.

Total monthly precipitation data for the Rincón station were obtained from the Puerto Rico and U.S. Virgin Islands Climatology Center at the University of Puerto Rico, Mayagüez Campus. The relationship between phenology and precipitation patterns was studied for this population.

Pollination – Pollination studies were carried out during August and September of 1997, for a total of twenty hours of observation, in order to determine the agents that pollinate *Buxus vahlii*. Shrubs were observed twice early in the morning (10 hrs.), once at noon during (5 hrs.) and once from the afternoon to the early evening (5 hrs.). No observations were carried during the night. Samples of potential pollinators were collected and identified.

<u>Germination experiments</u>

The requirements of light and humidity for germination and survivorship were tested in the Biology Greenhouse at the University of Puerto Rico, Mayagüez Campus. The experiment was conducted between October and December 1999. To avoid predation by rats, the experiment was carried out in three wire cages.

Solban shade cloth (73% shade) was used to simulate three different light intensities. One cage was covered with two layers to simulate a low light condition. Another was covered with a single layer to simulate a medium light condition. The third was left uncovered and used as a control with normal light intensity. Soil moisture was measured using a soil moisture meter (Lincoln Irrigation, Lincoln, Nebraska) by inserting one inch of the moisture meter probe into the soil. At each light intensity, three soil moisture conditions were established based on the moisture meter scale: dry (2-4), mesic (4-6) and wet (10).

Twenty-one pots were filled with commercial Sunshine mix # 4 and nine pots were filled with soil taken from the Rincón population area. In each pot, fifteen seeds were sown on the surface. Pots were placed inside plastic trays. Three pots with the commercial mixture and one pot of Rincón soil were identified for each soil moisture condition and placed inside each cage.

Soil moisture was checked every two days. Counts of germinated seeds were taken every two days. The seedlings were observed for three months in order to evaluate survival and growth.

Natural Impacts

Hurricanes – During the course of the present study, the category 2-3 Hurricane Georges (21-22 September 1998) crossed over the entire island of Puerto Rico from east to west. After the hurricane had passed, observations were made of its effects on the populations of *Buxus vahlii* in Rincón and Isabela.

Human impacts

Development – During January 1999, damage caused by the intended development of the area where the population near Frederiksted is located was found. Observations were made of human effects on B*uxus vahlii* in this population.

Illegal immigrants – Between 1997 and 1998, traces of the arrival of illegal immigrants and their passage through the Rincón population were observed on at least in five different occasions. Observations were made of the illegal immigrants' effects on B*uxus vahlii* in this population.

Fire – During April 1996, traces of fire were observed in the Rincón population. This was also seen during a visit in October of the same year. Observations were made of fire effects on B*uxus vahlii* in this population.

Introduced species – Cultivated and escaped plant species were observed growing in the Bayamón population locality. Observations were made of introduced species' effects on *Buxus vahlii* in this population.

Rock climbing – The hills where the Bayamón population is located are frequently used for rock climbing. Interviews with Science Park employees were done to investigate this kind of activity. Also, field observations were made in this population.

<u>Population genetics</u>

Electrophoretic studies require the availability of young leaves, as well as adequate facilities and reagents. During this study, several attempts were made to use electrophoresis techniques, and bands were obtained for the Rincón population, but results were not reliable. Therefore a morphometric study, which provides an estimate of the levels of intra- and interpopulational genetic variability, was performed.

Leaf morphometric studies were carried out for two populations in Puerto Rico (Rincón, Isabela) and one population in St. Croix (Frederiksted). A portion of one branch of each of fifty individuals from each population was collected haphazardly at around 1.5 m in height for the Rincón and Frederiksted populations and at four meters in height for the Isabela population, using accessibility and direct light exposure as criteria. Twenty-five adult leaves were removed from each branch, taped on a white paper sheet, numbered, pressed and left in a drying oven for four days. After drying they were scanned; subsequently length, width, and area were measured with the Sigma Scan Image (Jandel Scientific) program. The length: width ratio was calculated for each leaf. A one-way ANOVA was used to determine interpopulation differences. A nested ANOVA was used to evaluate variation among individuals within populations.

RESULTS

Distribution and abundance

There were six known *Buxus vahlii* populations: four in Puerto Rico: Rincón, Isabela and two in Bayamón (Figure 1) and two in St. Croix: Frederiksted and Christiansted (Figure 2). The populations are separated by distances of between 24 and 248 km (Table 1).

In Puerto Rico, *B. vahlii* was found in limestone soil. The Rincón population is found on the west side of a ravine, less than 1 km to the northeast of Punta Higüero, within 190 meters the shoreline (Municipality of Rincón, Barrio Puntas, 18°22’10’’N, 67°15’45’’W, 20-40 m elevation) (Figure 3). *Buxus vahlii* is a shrubby, understory species in this population. Table 2 shows some of the most common species associated with *B. vahlii* in this population. Associated canopy species are: *Ardisia obovata, Gymnanthes lucida, Bursera simaruba, Byrsonima crassifolia, Coccoloba diversifolia, Roystonea borinquena, Terminalia catappa, Calophyllum calaba, Plumeria alba, Coccoloba uvifera* and *Guettarda scabra*. Associated understory species are *Coccoloba microstachya, Comocladia glabra, Drypetes ilicifolia, Erithalis fruticosa, Leucaena leucocephala, Psycotria nervosa, Capparis flexuosa, Eugenia confusa, Tabebuia heterophylla, Thespesia grandiflora* and *Cecropia schreberiana*. Herbaceous species are *Vernonia cinerea* and *Oeceoclades maculata*. Climbing species and vines include *Smilax coriacea, Abrus precatorius, Cissampelos pareira* and *Passiflora suberosa.* This associated vegetation did not include any other rare or endangered species, but three species classified as critical elements according to the Natural Heritage Division of the DNER were found in this population: *Drypetes ilicifolia, Thespesia grandiflora* and *Erithalis fruticosa*.

The Isabela population is found on the east-facing slope of a very inclined ravine, 31 m south of the Juan Pérez footpath (Municipality of Isabela, Barrio Planas, 18°25'00''N, 16°58'30''W, 270-300 m elevation) (Figure 4). *Buxus vahlii* is an arborescent, canopy species in this population. Table 3 shows the most common species associated with *B. vahlii* in this population. Associated canopy species are *Coccoloba diversifolia* and *Coccoloba pubescens*. Associated understory species are *Ardisia obovata, Coccothrinax alta, Coccoloba microstachya* and *Myrica cerifera*. Herbaceous species are *Oncidium altissimum* and *Oeceoclades maculata*. Climbing species and vines include *Smilax domingensis* and *Vanilla claviculata.* An epiphytic species is *Tolumnia variegata*. There are no other rare or endangered plant species associated with *B. vahlii* in the area and only one species *Myrica cerifera* classified as a critical element was found in this population.

The Bayamón population that was studied is found on a hill located west-southwest of the entrance of the Sciences Park (Municipality of Bayamón, Barrio Hato Tejas, 18°24'20''N, 66°09'55'', 100 m elevation) from (Figure 5). *Buxus vahlii* is an arborescent, canopy species in this population. Table 4 shows the most common associated species associated with *B. vahlii* at this population. Associated canopy species are *Bursera simaruba, Coccoloba diversifolia*, *Tabebuia heterophylla*, *Bucida buceras*, *Clusia rosea, Cecropia schreberiana, Ficus lyrata, Coffea arabica, Zanthoxylum* *martinicense* and *Zanthoxylum monophyllum*. Associated understory species are *Comocladia glabra, Plumeria alba, Bourreria succulenta, Euphorbia leucocephala, Gymnanthes lucida, Jatropha curcas, Hibiscus rosa-sinensis, Pavonia spinifex, Leucaena leucocephala, Acacia retusa, Chiococca alba, Ixora coccinea, Murraya paniculata, Capsicum frutescens, Lantana camara* and *Piper aduncum*. Herbaceous species are *Gonolobus stephanotrichus, Bidens alba, Wedelia trilobata, Arthrostylidium sarmentosum, Bothriochloa pertusa, Urochloa maxima, Stachytarpheta jamaicensis, Bromelia pinguin, Oeceoclades maculata* and *Spathoglottis plicata*. Climbing species and vines are *Abrus precatorius, Rhynchosia reticulata, Cissampelos pareira, Cassytha filiformis, Tragia volubilis, Ipomoea tiliacea, Serjania polyphylla, Vanilla planifolia, Hylocereus trigonus* and *Smilax domingensis.* Epiphyte species are *Pitcairnia angustifolia, Anthurium crenatum, Epidendrum ciliare* and *Tectaria heracleifolia*. There were no other rare, endangered or critical species observed in this population.

In St. Croix, *B. vahlii* is also found on limestone-derived soil. The population near Frederiksted is located to the south of Sandy Point (Frederiksted town, 17°41'15''N, 64°53'00'', 20 m elevation) (Figure 6). *Buxus vahlii* is a shrubby, understory species in this population. Table 5 shows the most common associated species for this population. Associated canopy species are *Bursera simaruba, Tabebuia heterophylla, Canella winterana, Pisonia subcordata* and *Amyris elemifera*. Associated understory species are *Coccoloba diversifolia, Comocladia dodonea, Oplonia spinosa, Bourreria suculenta, Leucaena leucocephala, Pilosocereus royenii, Haematoxylum campechianum, Crossopetalum rhacoma, Argythamnia stahlii, Croton discolor, Gymnanthes lucida,* *Eugenia foetida, Rondeletia pilosa* and *Lantana involucrata*. An herbaceous species is *Scleria lithosperma*. A climbing species or vine is *Smilax domingensis.* An epiphytic species is *Tillandsia utriculata*. No rare or endangered species were found, but one critical element as defined by Puerto Rico was found in this population: *Rondeletia pilosa*.

The population near Christiansted was found to the southeast of the town (17°44’00’’N, 64°41’30’’W, 700 m elevation) (Figure 7). *Buxus vahlii* is a shrubby, understory species in this population. Table 6 shows the most common associated species for this population. Associated canopy species are *Tabebuia heterophylla, Amyris elemifera, Krugiodendron ferreum, Tecoma stans, Schaefferia frutescens, Bucida buceras, Piscidia carthagenensis* and *Zanthoxylum flavum*. Associated understory species are *Coccoloba microstachya, Comocladia dodonaea, Oplonia spinosa, Leucaena leucocephala, Capparis indica, Adelia ricinella, Croton astroides, Samyda dodecandra, Citharexylum fruticosum, Lantana camara, Solanum* sp.*, Calyptranthes* sp. and *Lantana involucrata*. Herbaceous species are *Urochloa maxima* and *Stachytarpheta jamaicensis*. Climbing species and vines are *Tournefortia volubilis, Ipomoea steudelii* and *Cucurbita* sp. No rare, endangered or critical species were found in this population.

<u>Population size and structure</u>

Individuals were tagged in Rincón and Isabela. In Rincón, the population was measured almost entirely by Kolterman and Breckon (1993). The total number of individuals does not appear to exceed seven hundred (personal observations). The basal diameter varied from 1 to 12 cm (n=630, mean=2.56 cm, s.d.=1.40) and the height varied from 0.3 to 5 m (n=630, mean=1.77 m, s.d.=0.89) in 1992. For the same population, the basal diameter ranged from 1 to 4.3 cm (n=52, mean=2.12, s.d.=0.11) and the height ranged from 0.7 to 5 m (n=52, mean=2.07, s.d.=0.14) during 1997. A total of 262 seedlings were counted in this locality during 1992 (Kolterman and Breckon, 1993).

There was a positive correlation between height and diameter in the Rincón population during 1992 ($r^2 = 0.321$; $p<0.001$) and during 1997 ($r^2= 0.575$; $p<<0.001$) data (Figures 8 and 9). There are significant differences for both height and diameter (Kruskal-Wallis test, P = 0.047; P = 0.038, respectively) in the Rincón population between 1992 and 1997.

In Isabela, a relatively small portion of the population was marked. The number of individuals possibly exceeds one thousand in this population (personal observations), making it one of the largest. The basal diameter ranged from 0.6 to 8.1 cm (n=168, mean=2.93 cm, s.d.=1.32) and the height ranged from 1.3 to 7 m (n=168, mean=3.40 m, s.d.=1.18). There was a positive correlation between height and diameter ($r^2= 0.582$; $p<<0.001$) in the Isabela population (Figure 10). Two hundred six seedlings were counted in this location.

In Bayamón, the entire population was measured. The total number of healthy, mature individuals found here was 21, making it the smallest population studied. Five cut individuals were observed, one of them with flowers and fruits. The basal diameter ranged from 2.2 to 8.9 cm (n=21, mean=4.70 cm, s.d.=2.09) and the height ranged from 1 to 6 m (n=21, mean=3.74 m, s.d.=1.51). There was a positive correlation between height and diameter ($r^2 = 0.59$, $p<0.001$) in the Bayamón population (Figure 11). Twenty-seven seedlings were counted at this location.

The population near Frederiksted appears to be the largest one, with more than one thousand individuals (personal observations). At the population near Christiansted, the limits were not determined, due principally to the difficult access to the population, the rainy season, and the tall grass that practically covers the *B. vahlii* individuals. By visual inspection, this population appears to be smaller than the population near Frederiksted.

Reproductive biology

Phenology - Phenological observations were made at the Rincón, Isabela and Bayamón populations. At Rincón, data were collected once in February, March and May 1998, three times in January and April 1998, and twice in all other months between June 1997 and May 1998. Sprouting of new leaves and branches was always observed on at least some plants during all the visits. During the year of study at the Rincón population, several patterns were observed. The period of greatest leaf production in terms of the presence of new leaves in *Buxus vahlii* (Figure 12) occured between June and August, followed by lesser leaf production during the months of September, October and November. During the rest of the study year leaf production was intermediate and showed small fluctuations. Bud formation in *B. vahlii* (Figure 13) showed two peaks, during April and August. Flowering activity in *B. vahlii* (Figure 14) showed one peak beginning in April, with the maximum activity during August and the minimum during November and December. There were two significant peaks of fruit production for *B. vahlii*, one in August and the other in November (Figure 15).

In Isabela and Bayamón the data recorded included only the reproductive status of the plants (buds, flowers and fruits). In the Isabela population data were collected during the months of August and September 1997. There were 15 individuals (9%) with at least one kind of reproductive structure. In Bayamón, population data were collected during October 2000. Twenty individuals (95%) were found with reproductive structures.

Pollination – Two species of insects were observed visiting the flowers of *B. vahlii*. The most abundant was the introduced honeybee, *Apis mellifera*, which was observed at all hours. Also, a small fly (*Dolichoderus* sp.) was observed visiting the flower early in the morning. Even though the presence of pollen was not observed on the collected specimens of insects, they cannot be discarded as possible pollinators of *B. vahlii*. Ants of the family *Lauxaniidae* were observed foraging on *B. vahlii* individuals.

Germination experiments

Germination experiments in Petri dishes were tried unsuccessfully during May 1997. Seeds were damaged by fungal infections.

Buxus vahlii showed a very low germination rate (Table 7). Only 6% of the seeds (25/450) succeeded in germinating. The highest proportion of germination (12%, 6/50) occurred under mesic conditions and total shade. Only 8% (2/25) of the seedlings survived for two months until the experiment finished. The surviving seedlings were in mesic and full light conditions.

Natural impacts

Hurricanes- The damage caused by Hurricane Georges in the Rincón population was minimal. *Buxus vahlii* was impacted by the wind, but defoliation and loss of branches occurred on only a few individuals. Following this event, overturned individuals were not observed. The major impact suffered by *B. vahlii* was the loss of reproductive structures (flowers and fruits). The canopy of much of the associated vegetation suffered defoliation and some broken branches. Two members of the associated vegetation of this population that did not occur before the hurricane (*Thespesia grandiflora* and *Cecropia schreberiana*) were observed growing in this population after the event.

Damage done by Hurricane Georges at Isabela was severe. Many trees were downed or toppled and the access to the population was very difficult. At the top of the ravine, there were gaps in the canopy and soil exposure. The major effect of the hurricane to *B. vahlii* was the loss of foliage. Effects of the hurricane in other populations were not studied.

Human impact

Development – In the population near Frederiksted, damage caused by the intended development of the area was observed during January 1999. The trails apparently caused by a bulldozer led directly to the entrance of the population. Some crushed *B. vahlii* shrubs were observed in a corner of the population.

Illegal immigrants – Between 1997 and 1998, traces of the arrival of illegal immigrants and their passage through the Rincón population were observed on at least five different occasions. This activity caused direct impact to the population. A variety of garbage (i.e., clothes, cigarette boxes, cans, papers, food envelopes, etc.) was found throughout the population area. Some of *B. vahlii* individuals were cut, presumably by the people involved in immigration activities in the Rincón area.

Fire – During April and October of 1996, the remnants of possible fires were observed in the Rincón population. The vegetation on the top of the hillside was the most affected by the fire. An area of approximately 10 m^2 was completely burned (all the *B. vahlii* individuals there died) and the surrounding vegetation was partially burned (*B. vahlii* presented stems burned at the top and partial loss of branches and leaves). During other visits, the remaining *B. vahlii* plants showed signs of regrowth from the base upwards, but remained dry at the top of the stem.

Introduced species – Seven cultivated and escaped plant species were found around the Bayamón population. These are: *Hibiscus rosa-sinensis*, *Ixora coccinea*, *Jatropha curcas*, *Murraya exotica*, *Vanilla planifolia*, *Coffea arabica* and *Capsicum frutescens*. One cultivated plant species (*Tecoma stans*) was found around the Christiansted population.

Rock climbing – During October 2000 in Bayamón, bolts used for rock climbing were observed at three sites on the vertical wall of the mogote, where the *B. vahlii* population is located. The employees of the Sciences Park confirmed the practice of this activity. A major trail that passed through the population was made, possibly to have better access to the hill. All five cut individuals (one with fruits) were near the trail. Six of the 27 seedlings counted in this population were in the middle of the trail. A variety of garbage (i.e., soda bottles, food envelopes, stewpots, etc.) was found at the entrance of the population area. Vandalism of *Bursera simaruba* trees was observed at the entrance of the population area. According to the employees this area was frequently used by drug users and students.

Population genetics

The electrophoresis technique was used and partial results were obtained (Table 8). Different combinations of systems and enzymes showed bands that could be used to determine genetic differentiation among populations.

In Table 9, the average leaf morphometric differences for the Rincón and Isabela populations were 1.1 cm (length), 0.3 cm (width), 1.8 cm^2 (area), and 0.3 (L/W); larger values were consistently observed in Rincón. The two *Buxus* populations from Puerto Rico were different for all leaf variables measured (Tables 10-13). The leaves of the Frederiksted and Isabela populations were similar (Tables 10-13). The average differences for these populations were: 0.2 cm (length), 0.1 cm (width), 0.4 cm^2 (area) and 0.1 (L/W) (Table 9); the leaves at Isabela were larger, but had a smaller L/W ratio. Finally, the Frederiksted and Rincón populations had different leaves (Tables 10-13). The average differences (Table 9) for these populations were: 1.3 cm (length), 0.4 cm (width), 2.2 cm^2 (area), and 0.2 (L/W); larger values were found for the Rincón population.

The nested ANOVA showed differences in all parameters for leaves in the three populations. These differences were found within: length (F=24.94), width (F=16.84), area (F=20.03) and L/W (F=12.15) and among populations: length (F=2774.17), width (F=848.77), area (F=2046.40) and L/W (F=199.80) Table 14.

DISCUSSION

The genus *Buxus* has undergone a remarkable evolutionary radiation within the Caribbean region. In the genus *Buxus*, adaptation to increasingly xerophytic environmental conditions seems to be involved with specialization to a range of edaphic conditions including siliceous, limestone and ultrabasic (serpentine) soils. Analysis of this phenomenon could explain the high rate of endemism and will contribute to a better understanding of the differentiation of this group of species (Köhler, 1981). In Puerto Rico, according to Liogier and Martorell (2000), there is one species of *Buxus* occurring mostly on serpentine soils (*Buxus portoricensis*) and another occurring on limestone (*Buxus vahlii*).

Buxus vahlii is endemic to Puerto Rico and St. Croix. Adams (1972) did not confirm it for Jamaica and, according to Köhler (1981), some specimens from that island labeled as *B. vahlii* differ fundamentally, indeed, in their pollen morphology. In Puerto Rico three populations (Rincón, Isabela and Bayamón) were confirmed with herbarium data. The possible existence of other populations in the south of Puerto Rico is not discarded. According to Liogier and Martorell (2000), *B. vahlii* is reported for Ponce. Vivaldi and Woodbury (1981) mention that a specimen collected by A. A. Heller and reported as being from the Ponce area is believed to have been mislabeled. Recently, *B. vahlii* has been identified as occurring in Guayanilla (José Sustache, personal communication). In St. Croix, the existence of two populations (near Frederiksted and near Christiansted) was confirmed. There were no data on additional populations on this island. Initially, the localization of *B.vahlii* suggested that this species was restricted to ravines and ledges. With the visit to the Frederiksted population it was confirmed that the species could also establish itself on plains.

The Rincón population is of medium size. This size should be large enough to resist minor disturbances in the area if the reproduction rate remains constant. The Rincón population showed smaller individuals with small diameters compared with the other populations (Figures 16-19). In Rincón, individuals are exposed to extreme conditions such as high winds and salt spray. At the Rincón population, comparisons between 1992 and 1997 data showed that recruitment occurred during this time, considering the heights and diameters found (Figures 20 and 21).

The Isabela population is one of the largest ones and is the only one located in a protected public forest.   In the Isabela population, *B. vahlii* individuals were taller and had a larger diameter (Figures 22 and 23). A thick layer of fallen *B. vahlii* leaves covers the floor of the forest, so the growth of seedlings and subsequent recruitment of new individuals is restricted by the availability of free patches of open soil. The Bayamón population is very small, very susceptible to extinction and located in an area of very high human impact. The population with the tallest individuals and the greatest basal diameter was in Bayamón (Figures 24 and 25). The population near Frederiksted is the largest one, and despite human impact has conserved a large number of individuals up to the present.

The average height and diameter of *Buxus vahlii* were smaller in the Rincón population than in Isabela and Bayamón populations. This may be attributed to the difference in the soil moisture conditions and light exposure between the localities. The plants at the Rincón population are subjected to high winds, salt spray and possibly a lack of canopy shading. Significant differences were found in height and diameter (Kruskal-Wallis test, P << 0.001, for both parameters) between the Rincón, Isabela and Bayamón populations.

Frankie et al. (1974), in a comparative phenological study of trees in the tropical wet and dry forests of the lowlands of Costa Rica, concluded that in both forests synchronization of flowering by many species appeared to be under the control of prevailing climatic conditions. In Rincón, and in the species in general, the production of new leaves extends through the entire year. *Buxus vahlii* exhibits very low bud presence and flowering activity during the dry season. The presence of fruits in *B. vahlii* showed an increment soon after the rainy season. The flowering time of individuals is known to have a genetic basis in some plant species (McIntyre and Best, 1978) and, by comparing fruit and seed production, the selection that favors individuals that flower earlier or later than others within a population can be discerned (Dieringer, 1991). Flowering occurs later during the year in Isabela and Bayamón populations than in Rincón. It was found, that reproduction occurs later in humid environments such as Isabela and Bayamón than in the drier environment of Rincón.

The evolution of floral diversity seems to be based upon specialized relationships with pollinators (Ollerton, 1996). *Buxus vahlii* flowers are unisexual; but the plants are monoecious and the inflorescences are bisexual. It might be that the inflorescence functions as a “flower” but this aspect was not studied. The masculine flowers are produced under the female flowers in a small terminal cluster. The three styles/stigmas of the female flower are relatively large and extend outside the flower. The flower morphology suggests that either wind or insects pollinate the plants. During this study, honeybees and flies visited *B. vahlii* flowers. Kolterman and Breckon (1993) mention that the lack of visitors to the flowers and the large number of fruits observed in *B. vahlii* strongly suggest the occurrence of autogamy (autopollination) because the taxa (families, genera) of angiosperms with unisexual flowers generally lack a self-incompatibility system.

Despite the small sample sizes, the greenhouse experiments suggest that seed germination occurs more frequently in shady and mesic conditions, but *B. vahlii* seedlings survive more successfully in full light conditions. These results are similar to those found in *Calliandra locoensis* Garcia & Kolterman (González-Rodríguez, 1998). Based on water use and crop coefficients, Schuch and Burger (1997) mentioned that *Buxus microphylla* var. *japonica* (Müll. Arg. ex Miq.) Rehd. & E. H. Wils. was an intermediate water user. Torres et al. (1996) describe *Buxus serpervirens* as frequent in rocky places and on slopes in limestone soils, where mesic conditions are maintained. There is a very little evidence for light as a factor influencing germination among cultivated plants. The seeds of most cultivated plants usually germinate equally well in the dark and in the light. In contrast, among wild plants much variability in behavior in response to light is observed. Daily illumination has also been shown to affect germination (Mayer and Poljakoff, 1989).

The impact of a hurricane on vegetation can be caused by wind, exposure, flooding, soil displacement, thunder storms and water stress following the hurricane. According to Miner (1997), damage caused to vegetation has been classified using an evaluation system of six categories: no damage, defoliation, loss of small branches, loss of large branches, toppled trunks, and overturned trees (exposed roots). The major damage caused by Hurricane Georges in *Buxus vahlii* was defoliation, which was more severe in the Isabela population. There, *B. vahlii* trees grow taller than in the Rincón population, where they are part of the understory and may be more protected. In a comparison of storm damages caused by Hurricane Georges to 24 species of trees in Puerto Rico, tall trees were subjected to greater wind force than small trees, and some species suffered more damage than others. Greater strength of the wood in roots, trunk and branches impart resistance to tipping over, and flexibility causes a large reduction in cross-sectional area exposed to the wind, and thus reduces drag (Francis, 2000). *Buxus vahlii* populations did not suffer great impact by Hurricane Georges in comparison with effects on other native species in the areas, so this species appears to have developed resistance to such events. *Buxus vahlii* can be considered adapted to that kind of event. The presence of two new species following the hurricane increases the diversity of the vegetation; their possible effects on *B. vahlii* are unknown, but since they are successional species they can disappear with the reestablish canopy.

Although all the three populations in Puerto Rico are on public land, only the Isabela population is in a Commonwealth forest managed by the DNER. Humans are still present in a preserved system and exert their influence both directly and indirectly. Despite legal protection, the greatest threat to rare plant populations in most preserve settings is anthropogenic (Power-Bratton and White, 1980).

The *B. vahlii* population that has received the greatest impact of development is the population near Frederiksted. This impact was direct and was apparently caused by a bulldozer. At present, there are efforts being made by the USFWS to buy the land where this population is located in order to preserve it (Susan Silander, personal communication). This action would prevent future destruction of the area and offer *B. vahlii* a chance to recover.

A portion of the Bayamón population was destroyed by limestone mining several years ago (Vivaldi and Woodbury, 1981). At present, the Bayamón population is on a mogote that was partially destroyed to make room for a commercial building. This action has increased human impact to the population by promoting the construction and use of alternative trails facilitating access to the population.

The possible entrance of illegal immigrants in the Rincón population was first mentioned by Kolterman and Breckon (1992). They report that, according to local people, illegal immigrants use the site to hide after landing. The existence of this activity is confirmed by considering the type of garbage found at the population site. This activity has caused direct impact to the population, by the individuals who hide in the area, the people who promote the business of illegal immigration and the federal officials who attempt to capture and deport them.

Plant populations respond in remarkably diverse ways to fire. Some remain essentially stable from one fire to the next. Others may fluctuate from explosive growth to extinction (Keeley, 1987; Bond and van Wilgen, 1994; Reyes et al., 1997; Tárrega et al., 1997; Herranz, 1998). The responses to a given fire depend on its particular characteristics; fire is not a consistent phenomenon but comprises several variable characteristics including intensity, season, frequency and extent (Whelan, 1995; Segura et al., 1998). A low-intensity fire occurred at the Rincón population but, due to the high aggregation of *B. vahlii* individuals (Hopkins test; h= 6.06), the population was severely impacted. Threats to native species from exotics are particularly evident in urban settings. One example is the exclusion of forest floor species (Anderson, 1999).

The lack of forests in urban ecosystems promotes the use of the remaining green patches for different purposes and creates several impacts to those ecosystems (Rowntree, 1998). Without proper supervision, the sustainability of the areas can be exceeded. The mogote where the Bayamón population is located offers the people of the area an alternative to come into contact with nature. Also, the presence of bolts in the area promotes the activity of rock climbing. A total of 25 *B. vahlii* individuals were previously reported for the Bayamón population (USFWS, 1987). At present, 26 adult individuals were observed, five of which were cut. For such a population, the loss of a single individual is very significant because, over a period of 13 years, there is no indication of any recent recruitment of adult trees in this area. Smaller populations are more likely than large ones to go to extinct, for a variety of reasons (Menges, 1991).

Considering the distance between the populations (Table 1), the relatively small flowers and the apparently limited dispersiveness of the seeds, the existence of gene exchange between them is unlikely.

Notwithstanding their geographical isolation, the differences between the Isabela and Frederiksted populations were not significant for any of the variables measured. On the other hand, significant differences were found for all the parameters measured between the population of Rincón and each of the other two. These differences between populations could be indicative of the possible influences of the different ecological factors prevailing among the studied areas, which are rainfall, light exposure and wind. In the case of L/W, genetic differences as well as ecological factors among populations are probably involved.

A study of the two remaining populations in Bayamón and near Christiansted is suggested, to compare them with the Rincón, Isabela and Frederiksted populations. The use of electrophoretic analysis or DNA studies is also recommended for further clarification regarding the genetic relationships among these populations.

STATUS AND RECOVERY

The *Buxus vahlii* recovery plan provides guidance for reversing the decline of *B. vahlii* and restoring the species to a stable, secure, and self-sustaining status, thereby permitting it to be reclassified from endangered to threatened, and perhaps eventually allowing its removal from the federal list (USFWS, 1987). Based on this research, some aspects of the recovery plan have to be updated.

To prevent further habitat loss and population decline, habitat and plants should be protected. Major efforts should be made to develop suitable alternatives for site protection through public or private agencies. Only three out of five populations are on public property (Rincón, Isabela and Bayamón). Efforts to buy the Frederiksted population site have been completed by USFWS, but the Christiansted's population site is still on private property.

A representative sample of *B. vahlii* individuals was monitored in the three populations studied in Puerto Rico. The general health and reproductive status of individuals in each population were established based on field observations and data analysis.

Changes in population sites (natural or human related) and their impacts on individuals were observed for *B. vahlii* in all populations. The greatest threat confronted by *B. vahlii* was direct and indirect human impact. This has affected at least three populations of *B. vahlii* (Rincón, Bayamón and Frederiksted). The other two populations (Isabela and Christiansted) were less affected by human impact, since one of them was located in a protected area and both were located in places with poor accessibility.

Management measures such as fencing, labeling, signs implementation and general public education should be taken immediately in order to preserve the most impacted populations.

The Commonwealth's 1985 Regulation to Govern the Management of Threatened and Endangered Species provides for criminal penalties for illegal collection of or damage to listed plant species, regardless of land status. *Buxus vahlii* is on the Commonwealth's list, and the regulation must be enforced with regard to this species. Federal and Commonwealth conservation agencies should educate the public on plant conservation values and regulations. In Rincón, a community education plan should be inplemented (i.e., conferences, signs in adjacent areas, demarcation of the whole area). Communication between agencies is necessary for the implementation of existing laws. An inter-agency effort between the USFWS and immigration-related agencies should be developed to enforce the existing immigration laws. In Bayamón, a sociological study of the people that live and visit the population site should be done. Based on that study, a management plan that offers alternatives and promotes community efforts should be developed and implemented. The bolts used for rock climbing should be removed immediately. In Frederiksted, the acquisition of the population area should be completed, in order to avoid the further development of the area, which would otherwise end in the total destruction of the population.

Decisions regarding management of existing populations and recovery priorities will be affected by the species' abundance and additional biological or ecological information. This study provides the required information for the Rincón, Isabela, Bayamón and Frederiksted populations.

Three additional populations have to be included in the recovery plan (Isabela, Frederiksted and Christiansted). The existence of new populations of *B. vahlii* in Guayanilla also has been verified. New potential sites should be identified and inventoried. The karst region of Puerto Rico is sufficiently rugged that likelihood of undiscovered *B. vahlii* populations remains high.

Buxus vahlii habitat requirements such as associated plant species and soil type were defined based on the populations studied. Qualitative and quantitative data on site conditions were obtained for studied populations; this information can be used as a guide for species management decisions.

The scarcity of knowledge on the reproductive biology of this species limits management of existing populations and delays the establishment of new populations.

Information of reproductive biology (flowering frequency, pollinators, etc.) was studied in Rincón population for one year. The reproductive status of Isabela and Bayamón populations were obtained during field visits. Additional studies regarding pollination mechanisms and seed production and dispersal are recommended.

Buxus vahlii showed a very low germination rate. Germination requirements were studied under different light and soil moisture conditions. *Buxus vahlii* exhibited higher germination rates under shade and mesic conditions. Seedlings survived under full light and mesic conditions. The evaluation of seedling establishment, growth, and the feasibility of artificial propagation is recommended for future studies.

A selection of appropriate sites for population enhancement and reintroduction using artificially propagated material is necessary. Considering the small number of individuals and the high human impact, reintroduction of *B. vahlii* is highly recommended for the Bayamón population. The success and ecological appropriateness of planting or transplanting propagative material depends upon adequate consideration of geography and habitat. *Buxus vahlii* occurs primarily in inclined limestone ravines.

Habitat protection should be a priority vs. propagation and reintroduction. Conservation of the known populations is strongly recommended, because there are genetic differences between and among populations. All the populations are presumed to be locally adapted to specific site conditions. The number of populations and individuals necessary to ensure the species' stability, security, and self-perpetuation depends on a management plan that guarantees the protection of the species. Once the management recommendations have been completed *Buxus vahlii* can be remove from the endangered species list.

CONCLUSIONS

Based on the present work, the following conclusions can be stated:

1) *Buxus vahlii* is known from five populations, three in Puerto Rico (Rincón, Isabela and Bayamón) and two in St. Croix (Frederiksted and Christiansted).

2) New leaf production occurs throught the year, but sexual reproduction is seasonal, occurring primarily during the rainy season.

3) Seed germination requires moist conditions, but growth and flowering are promoted by sun-exposed conditions. Long-term shade conditions are not suitable for seedlings of *B. vahlii*, and shaded conditions limit the species' range in the different population localities.

4) The major threat to *B. vahlii* is human impact caused by development, fire, illegal immigrants, introduced species and rock climbing.

5) Hurricanes do not represent a threat to *B. vahlii.*

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TABLES

Table 1. Approximate distance between all the *Buxus vahlii* populations. Numbers are in kilometers.

	Rincón	Isabela	Bayamón	Fredriksted	Christiansted
Rincón	--	24	120	224	248
Isabela	24	--	96	200	216
Bayamón	120	96	--	104	120
Fredriksted	224	200	104	--	16
Christiansted	248	216	120	16	--

Table 2. List of plant species associated with *Buxus vahlii* at the Rincón locality.

Family	Species
Anacardiaceae	*Comocladia glabra* (Schultes) Spreng.
Apocynaceae	*Plumeria alba* L.
Arecaceae	*Roystonea borinquena* O. F. Cook
Asteraceae	*Vernonia cinerea* (L.) Less.
Bignoniaceae	*Tabebuia heterophylla* (DC.) Britt.
Burseraceae	*Bursera simaruba* (L.) Sarg.
Campanulaceae	*Hippobroma longifolia* (L.) G. Don
Capparaceae	*Capparis flexuosa* (L.) L.
Clusiaceae	*Calophyllum calaba* L.
Combretaceae	*Terminalia capata* L.
Euphorbiaceae	*Drypetes ilicifolia* Krug & Urb. *Gymnanthes lucida* Sw.
Malpighiaceae	*Byrsonima crassifolia* (L.) Sarg.
Malvaceae	*Thespesia grandiflora* DC.
Menispermaceae	*Cissampelos pareira* L.
Mimosaceae	*Leucaena leucocephala* (Lam.) De Wit
Moraceae	*Cecropia schreberiana* Miq.
Myrsinaceae	*Ardisia obovata* Desv. ex Ham.
Myrtaceae	*Eugenia confusa* DC.

Table 2. (cont.)

Family	Species
Orchidaceae	*Oeceoclades maculata* (Lindl.) Lindl.
Papilionaceae	*Abrus precatorius* L.
Passifloraceae	*Passiflora suberosa* L.
Polygonaceae	*Coccoloba diversifolia* Jacq.
Polygonaceae	*Coccoloba microstachya* Willd. *Coccoloba uvifera* (L.) L.
Rubiaceae	*Erithalis fruticosa* L. *Guettarda scabra* (L.) Vent. *Psychotria nervosa* Sw.
Smilacaceae	*Smilax coriacea* Spreng.

Table 3. List of plant species associated with *Buxus vahlii* at the Isabela locality.

Family	Species
Arecaceae	*Coccothrinax alta* (O.F. Cook) Becc.
Myricaceae	*Myrica cerifera* L.
Myrsinaceae	*Ardisia obovata* Hamilt.
Myrtaceae	*Eugenia* sp.
Orchidaceae	*Oeceoclades maculata* (Lindl.) Lindl.
	Oncidium altissimum (Jacq.) Swartz
	Tolumnia variegata (Swartz) Braem
	Vanilla claviculata (W. Wright) Swartz
Polygonaceae	*Coccoloba diversifolia* Jacq.
	Coccoloba microstachya Willd.
	Coccoloba pubescens L.
Smilacaceae	*Smilax domingensis* Willd.

Table 4. List of plant species associated with *Buxus vahlii* at the Bayamón locality.

Family	Species
Anacardiaceae	*Comocladia glabra* (Schultes) Spreng.
Apocynaceae	*Plumeria alba* L.
Araceae	*Anthurium crenatum* (L.) Kunth.
Asclepiadaceae	*Gonolobus stephanotrichus* Griseb.
Asteraceae	*Bidens alba* (L.) DC. *Wedelia trilobata* (L.) Hitchc.
Bignonaceae	*Tabebuia heterophylla* (DC.) Britt.
Boraginaceae	*Bourreria succulenta* Jacq.
Bromeliaceae	*Bromelia pinguin* L. *Pitcairnia angustifolia* Aiton
Burseraceae	*Bursera simaruba* (L.) Sarg.
Cactaceae	*Hylocereus trigonus* (Haw.) Saff.
Clusiaceae	*Clusia rosea* Jacq.
Combretaceae	*Bucida buceras* L.
Convolvulaceae	*Ipomoea tiliacea* (Willd.) Choisy ex DC.
Euphorbiaceae	*Euphorbia leucocephala* Lotsy *Gymnanthes lucida* Sw. *Jatropha curcas* L. *Tragia volubilis* L.
Lauraceae	*Cassytha filiformis* L.
Malvaceae	*Hibiscus rosa-sinensis* L.

Table 4. (cont.)

Family	Species
Malvaceae	*Pavonia spinifex* (L.) Cav.
Menispermaceae	*Cissampelos pareira* L.
Mimosaceae	*Acacia retusa* (Jacq.) Howard
	Leucaena leucocephala (Lam.) De Wit
Moraceae	*Cecropia schreberiana* Miq.
	Ficus lyrata Warb.
Orchidaceae	*Epidendrum ciliare* L.
	Oeceoclades maculata (Lindl.) Lindl.
	Spathoglottis plicata Blume
	Vanilla planifolia Jacks.
Papilionaceae	*Abrus precatorius* L.
	Rhynchosia reticulata (Sw.) DC.
Piperaceae	*Piper aduncum* L.
Poaceae	*Arthrostylidium sarmentosum* Pilg.
	Bothriochloa pertusa (L.) A. Camus
	Urochloa maxima (Jacq.) R.D.Webster
Polygonaceae	*Coccoloba diversifolia* Jacq.
Polypodiaceae	*Tectaria heracleifolia* (Willd.) Underw.
Rubiaceae	*Chiococca alba* (L.) Hitchc.
	Coffea arabica L.
	Ixora coccinea L.
Rutaceae	*Murraya paniculata* (L.) Jack
	Zanthoxylum martinicense (Lam.) DC.
	Zanthoxylum monophyllum (Lam.) P. Wilson

Table 4. (cont.)

Family	Species
Sapindaceae	*Serjania polyphylla* (L.) Radlk.
Smilacaceae	*Smilax domingensis* Willd.
Solanaceae	*Capsicum frutescens* L.
Verbenaceae	*Lantana camara* L. *Stachytarpheta jamaicensis* (L.) Vahl.

Table 5. List of plant species associated with *Buxus vahlii* at the Frederiksted locality.

Family	Species
Acanthaceae	*Oplonia spinosa* (Jacq.) Raf.
Anacardiaceae	*Comocladia dodonea* (L.) Urb.
Bignoniaceae	*Tabebuia heterophylla* (DC.) Britt.
Bromeliaceae	*Tillandsia utriculata* L.
Boraginaceae	*Bourreria succulenta* Jacq.
Burseraceae	*Bursera simaruba* (L.) Sarg.
Cactaceae	*Pilosocereus royenii* (L.) Byles & Rowley
Caesalpinaceae	*Haematoxylum campechianum* L.
Canellaceae	*Canella winterana* (L.) Gaertn.
Celastraceae	*Crossopetalum rhacoma* Crantz
Cyperaceae	*Scleria lithosperma* (L.) Sw.
Euphorbiaceae	*Argythamnia stahlii* Urb. *Croton discolor* Willd. *Gymnanthes lucida* Sw.
Mimosaceae	*Leucaena leucocephala* (Lam.) De Wit
Myrtaceae	*Eugenia foetida* Pers.
Nyctaginaceae	*Pisonia subcordata* Sw.
Polygonaceae	*Coccoloba diversifolia* Jacq.
Rubiaceae	*Rondeletia pilosa* Sw.

Table 5. (cont.)

Family	Species
Rutaceae	*Amyris elemifera* L.
Smilacaceae	*Smilax domingensis* Willd.
Verbenaceae	*Lantana involucrata* L.

Table 6. List of plant species associated with *Buxus vahlii* at the Christiansted locality.

Family	Species
Acanthaceae	*Oplonia spinosa* (Jacq.) Raf.
Anacardiaceae	*Comocladia dodonea* (L.) Urb.
Bignoniaceae	*Tabebuia heterophylla* (DC.) Britt. *Tecoma stans* (L.) Juss. ex HBK.
Boraginaceae	*Tournefortia volubilis* L.
Capparaceae	*Capparis indica* (L.) Fawc. & Rendle
Celastraceae	*Schaefferia frutescens* Jacq.
Combretaceae	*Bucida buceras* L.
Convolvulaceae	*Ipomoea steudelii* Millsp.
Cucurbitaceae	*Cucurbita* sp.
Euphorbiaceae	*Adelia ricinella* L. *Croton astroides* Dryand.
Flacourtiaceae	*Samyda dodecandra* Jacq.
Mimosaceae	*Leucaena leucocephala* (Lam.) De Wit
Myrtaceae	*Calyptranthes* sp.
Papilionaceae	*Piscidia carthagenensis* Jacq.
Poaceae	*Urochloa maxima* (Jacq.) R.D.Webster
Polygonaceae	*Coccoloba microstachya* Willd.
Rhamnaceae	*Krugiodendron ferreum* (Vahl) Urb.
Rutaceae	*Amyris elemifera* L.

Table 6. (cont.)

Family	Species
Rutaceae	*Zanthoxylum flavum* Vahl.
Solanaceae	*Solanum* sp.
Verbenaceae	*Citharexylum fruticosum* L. *Lantana camara* L. *Lantana involucrata* L. *Stachytarpheta jamaicensis* (L.) Vahl.

Table 7. Seed germination and seedling survivorship of *Buxus vahlii* at three levels of soil moisture and light exposure. G = number of seeds that germinated; S = number of seedlings that survived for two months.

	Light level			
Moisture level	Full light G/S	Semi-shade G/S	Shade G/S	Total
Dry	1/0	1/0	4/0	6/0
Mesic	5/2	1/0	6/0	12/2
Wet	3/0	0/0	4/0	7/0
Total	9/2	2/0	14/0	

Table 8. Presence/absence of electrophoretic bands using different combinations of systems and enzymes following Hamrick's format (n=6).

Systems	4 Camellia	4 Cecropia	10 Camellia	34 Camellia	34 Morden	34 Needle
Enzymes						
AAI	n/a	n/a	n/a	positive	n/a	n/a
CE	negative	n/a	n/a	n/a	positive	n/a
DIA	n/a	n/a	n/a	negative	n/a	n/a
GDH	n/a	n/a	n/a	negative	n/a	negative
IDH	n/a	negative	positive	negative	n/a	n/a
MDH	n/a	negative	n/a	n/a	n/a	n/a
ME	negative	n/a	n/a	n/a	negative	n/a
MNR	n/a	n/a	n/a	positive	n/a	positive
PGI	n/a	negative	positive	negative	n/a	negative
PGIA	n/a	n/a	negative	n/a	n/a	n/a
PGIL	n/a	n/a	n/a	n/a	n/a	positive
PGM	n/a	positive	negative	positive	n/a	negative
SKDH	negative	n/a	n/a	n/a	positive	n/a
SKIK	n/a	n/a	positive	n/a	n/a	n/a
SKMK	n/a	n/a	n/a	positive	n/a	n/a
TPI	n/a	n/a	n/a	negative	n/a	n/a
6-P	positive	n/a	n/a	n/a	positive	n/a

Table 9. Morphometric differences among populations.

	Length (cm)	Width (cm)	Area (cm^2)	L/W
Frederiksted	3.2 ± 0.59	1.1 ± 0.25	2.3 ± 0.88	2.9 ± 0.47
Rincón	4.5 ± 0.85	1.5 ± 0.38	4.5 ± 1.76	3.1 ± 0.64
Isabela	3.4 ± 0.51	1.2 ± 0.22	2.7 ± 0.79	2.8 ± 0.43

Table 10. One-way ANOVA data for leaf length.

Source	DF	SS	MS	F	P
Factor	2	1240.7	620.3	1366.2	<0.001
Error	3494	1586.5	0.5		
Total	3496	2827.2			

Table 11. One-way ANOVA data for leaf width.

Source	DF	SS	MS	F	P
Factor	2	84.8	42.4	497.1	<0.001
Error	3494	297.9	0.1		
Total	3496	382.7			

Table 12. One-way ANOVA data for leaf L/W.

Source	DF	SS	MS	F	P
Factor	2	75.0	37.5	135.3	<0.001
Error	3494	968.6	0.3		
Total	3496	1043.6			

Table 13. One-way ANOVA data for leaf area.

Source	DF	SS	MS	F	P
Factor	2	3427.0	1713.5	1110.9	<0.001
Error	3494	5389.1	1.54		
Total	3496	8816.1			

Table 14. Nested ANOVA data for leaf parameters.

Parameter	F	P
Length		
Among populations	2774.17	< 0.001
Within populations	24.94	< 0.001
Width		
Among populations	848.77	< 0.001
Within populations	16.84	< 0.001
Area		
Among populations	2046.4	< 0.001
Within populations	20.03	< 0.001
L/W		
Among populations	199.8	< 0.001
Within populations	12.15	< 0.001

FIGURES

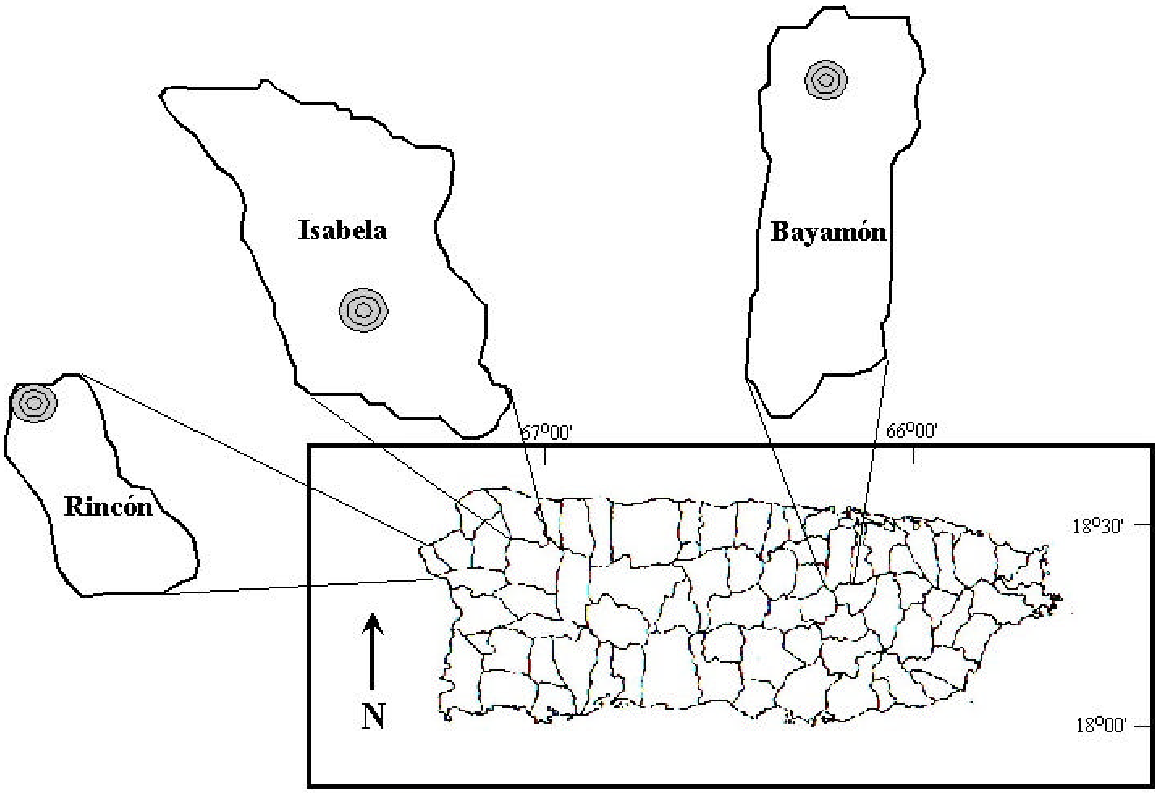

Figure 1. Map showing the location of *Buxus vahlii* in Puerto Rico.

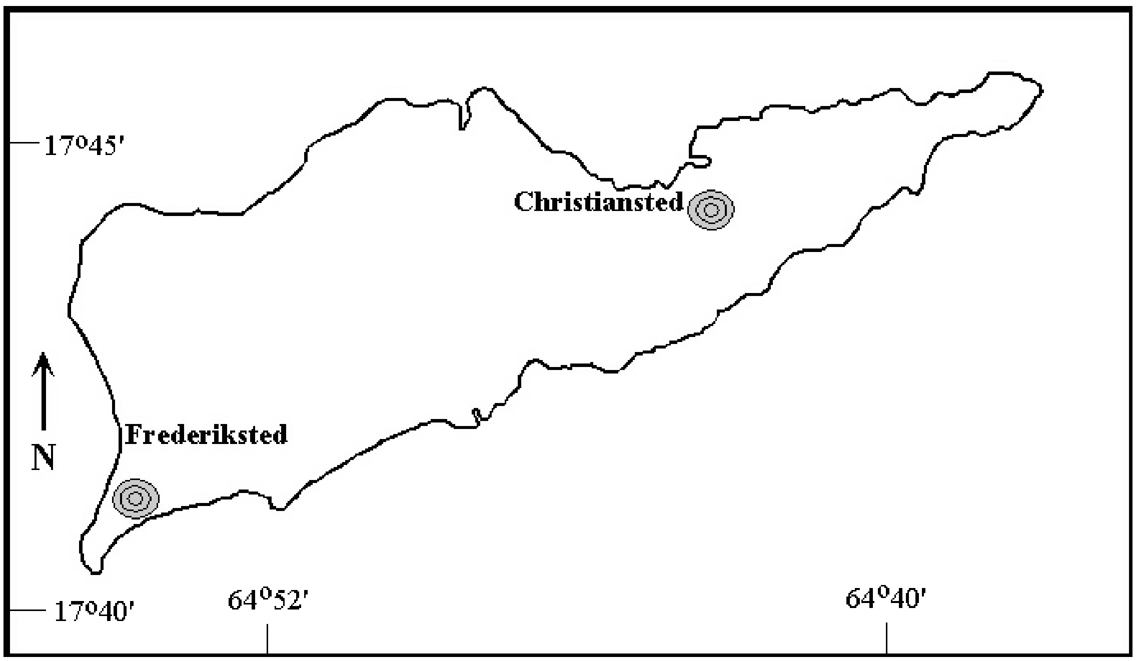

Figure 2. Map showing the location of *Buxus vahlii* in St. Croix.

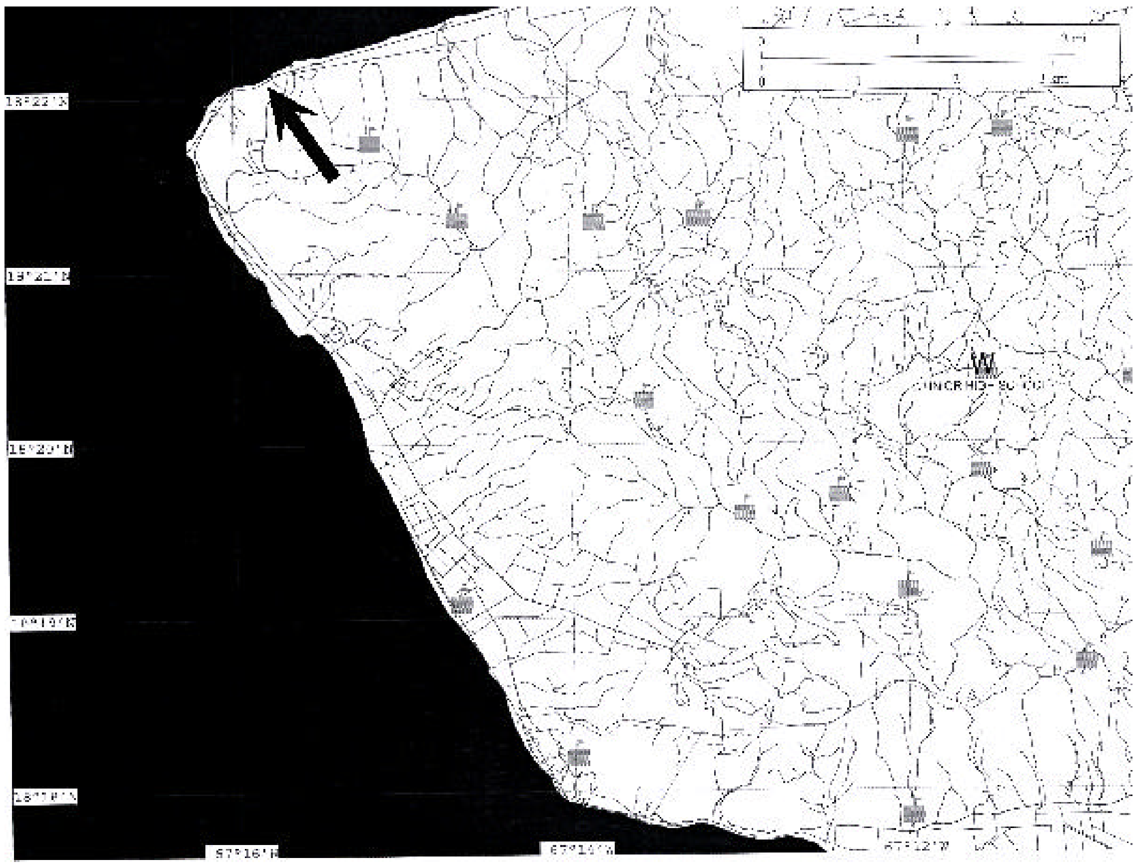

Figure 3. Location of the population of *Buxus vahlii* in Rincón.

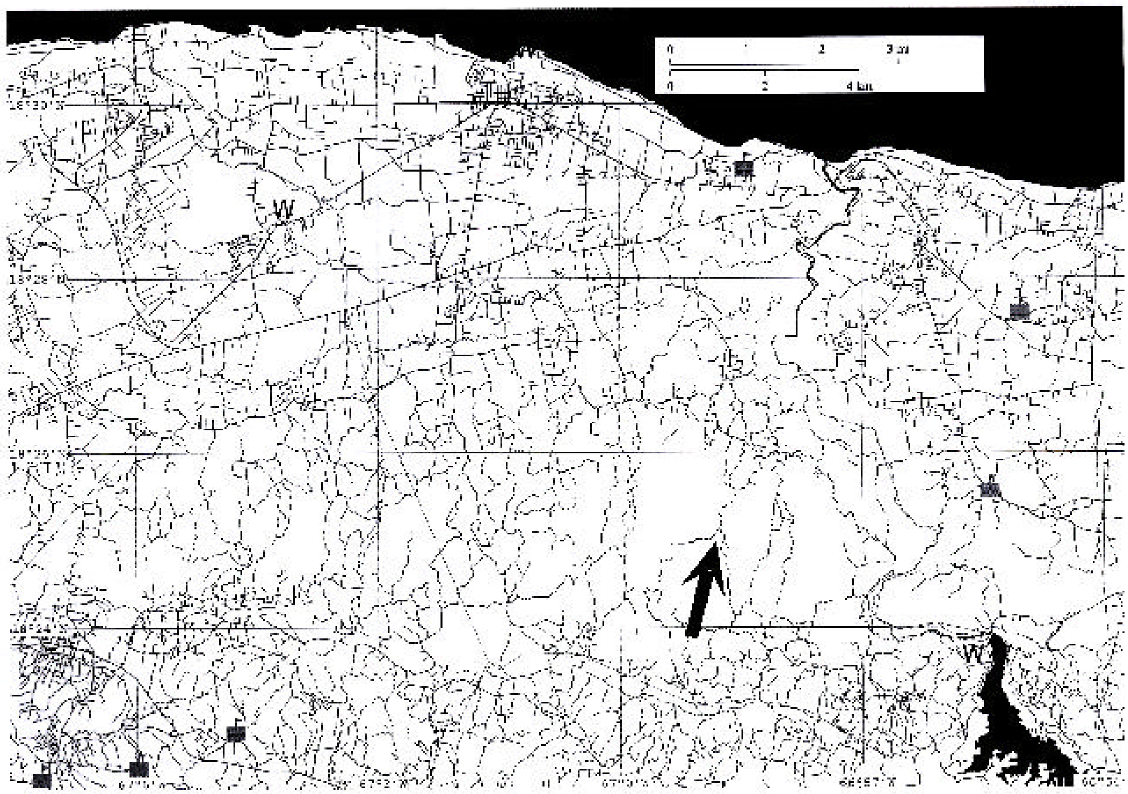

Figure 4. Location of the population of *Buxus vahlii* in Isabela.

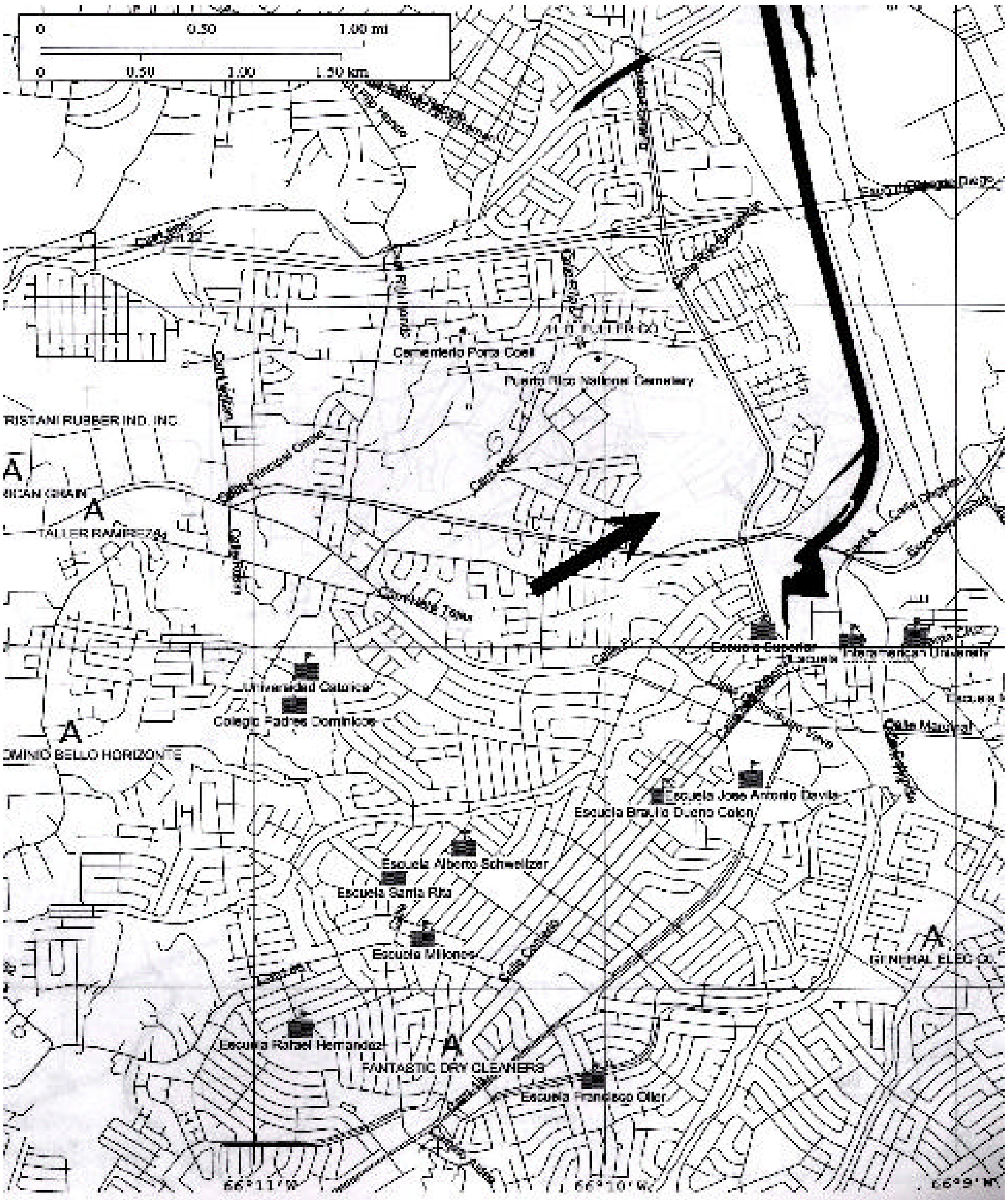

Figure 5. Location of the population of *Buxus vahlii* in Bayamón.

Figure 6. Location of the population of *Buxus vahlii* in Fredriksted.

Figure 7. Location of the population of *Buxus vahlii* in Christiansted.

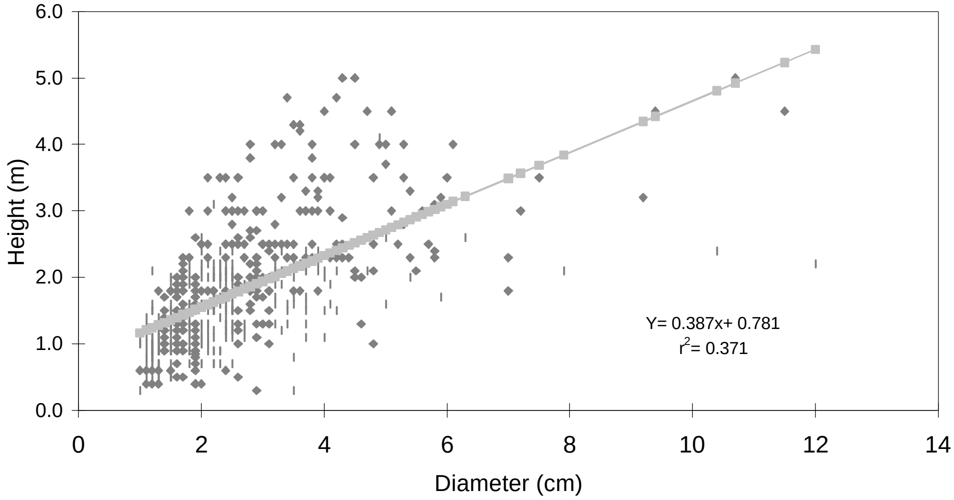

Figure 8. Relation between basal diameter and height of *Buxus vahlii* measured at the Rincón population during 1992.

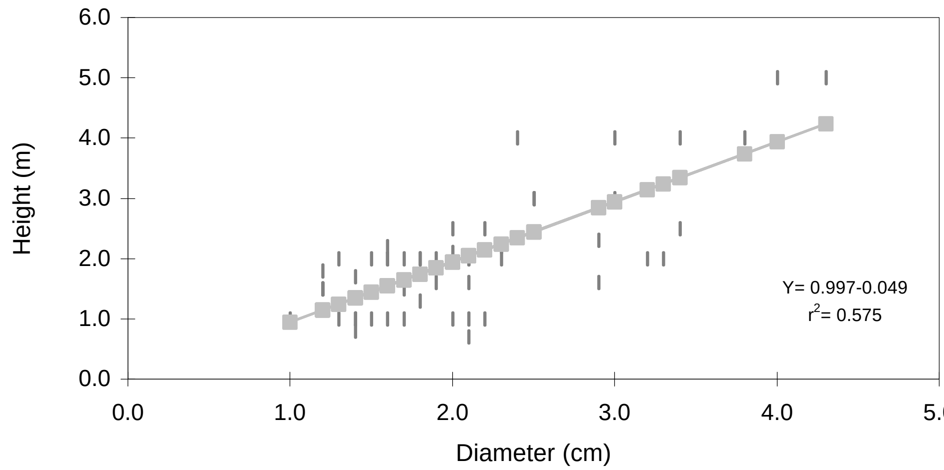

Figure 9. Relation between diameter and height of *Buxus vahlii* measured at the Rincón population during 1997.

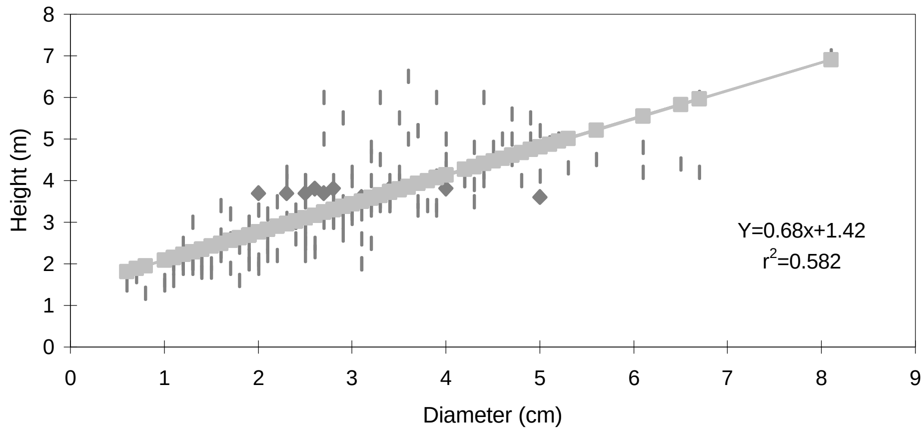

Figure 10. Relation between diameter and height of *Buxus vahlii* measured at the Isabela population.

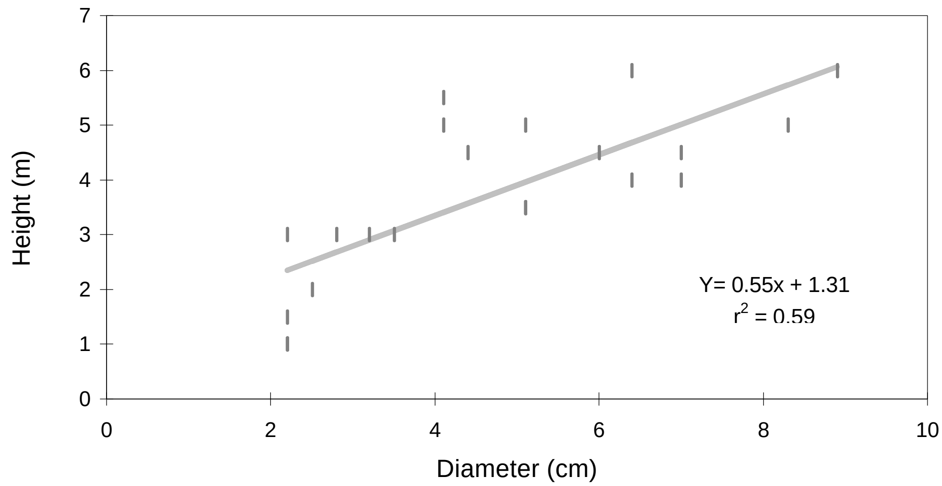

Figure 11. Relation between diameter and height of *Buxus vahlii* measured at the Bayamón population.

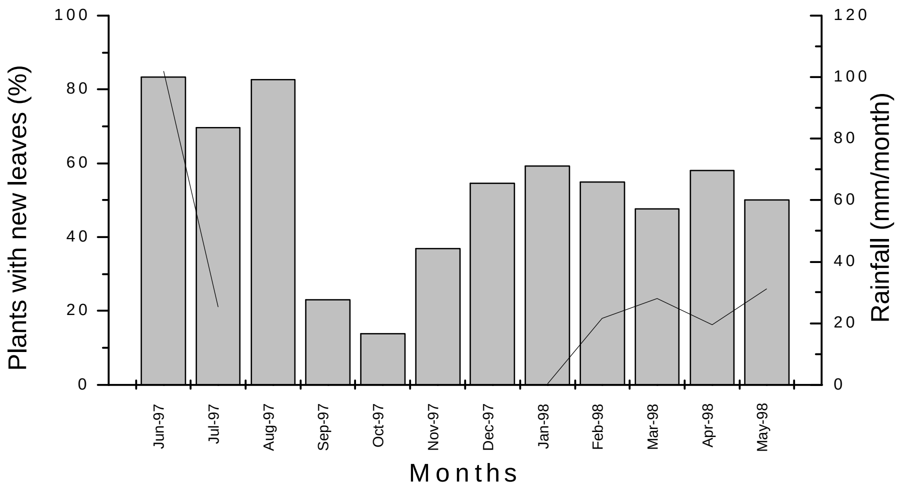

Figure 12. Relation between plants with new leaves and rainfall for *Buxus vahlii* at the Rincón population. Rainfall data between August and December were not available.

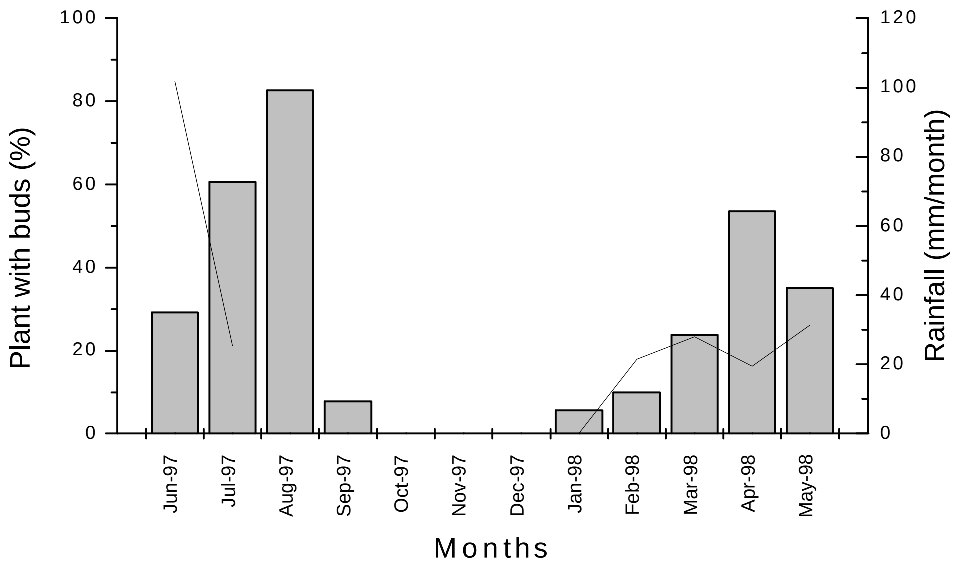

Figure 13. Relation between plants with buds and rainfall for *Buxus vahlii* at the Rincón population. Rainfall data between August and December were not available.

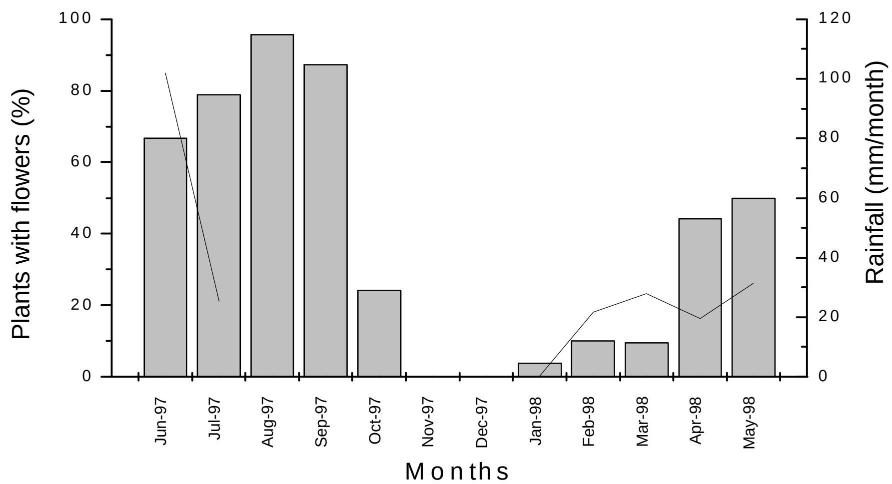

Figure 14. Relation between plants with flowers and rainfall for *Buxus vahlii* at the Rincón population. Rainfall data between August and December were not available.

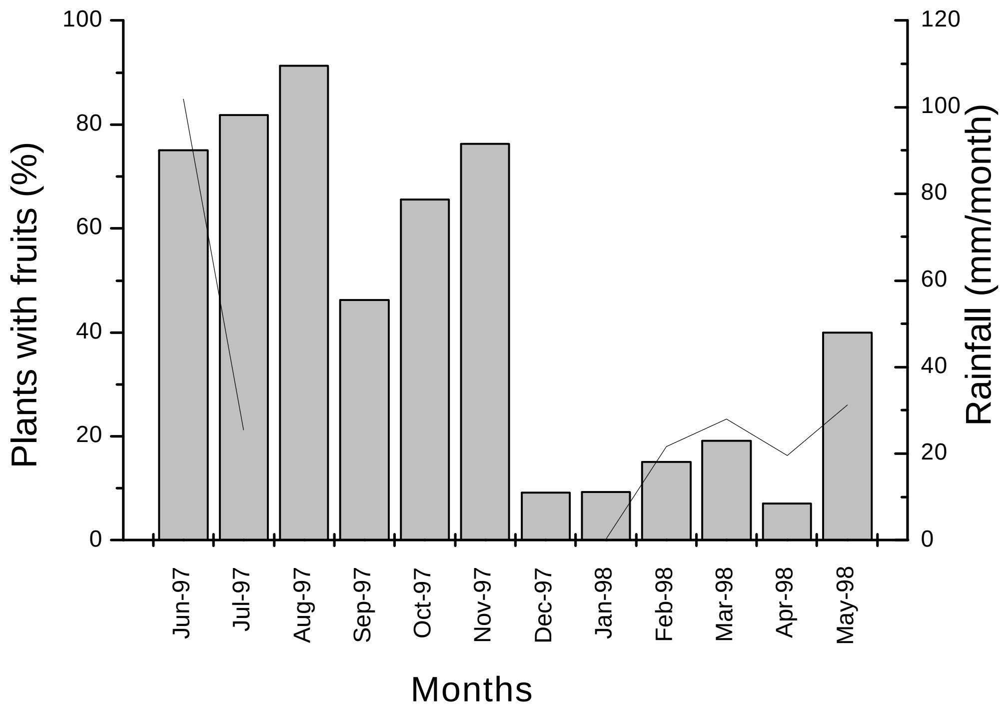

Figure 15. Relation between plants with fruits and rainfall for *Buxus vahlii* at the Rincón population. Rainfall data between August and December were not available.

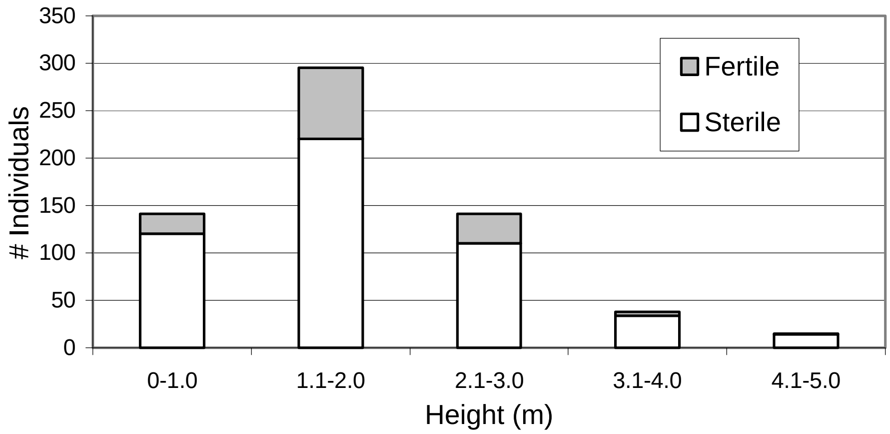

Figure 16. Distribution of height vs. reproductive status for all individuals of *Buxus vahlii* measured at the Rincón population during March, September, and October 1992 and March1993.

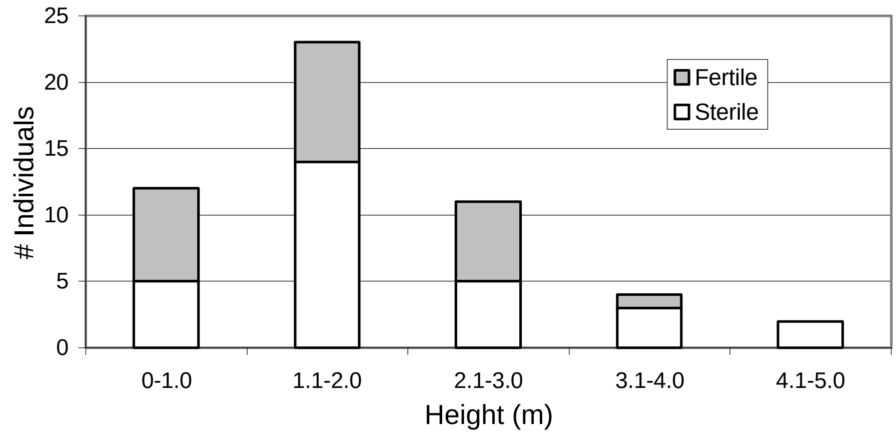

Figure 17. Distribution of height vs. reproductive status for all individuals of *Buxus vahlii* measured at the Rincón population during August 1997.

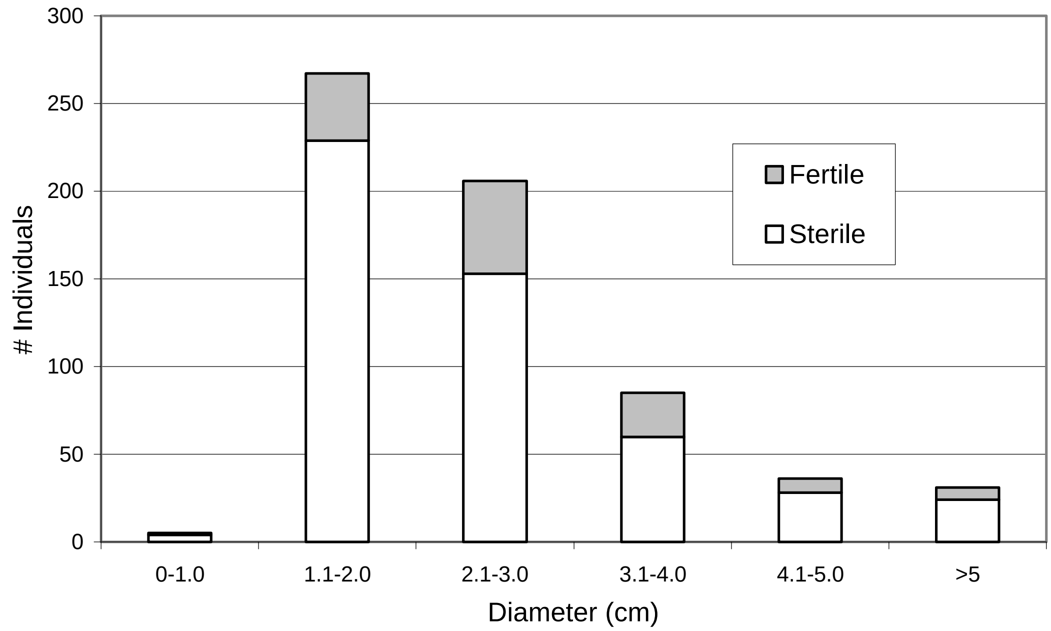

Figure 18. Distribution of basal diameter vs. reproductive status for all individuals of *Buxus vahlii* measured at the Rincón population during March, September, and October 1992 and March1993.

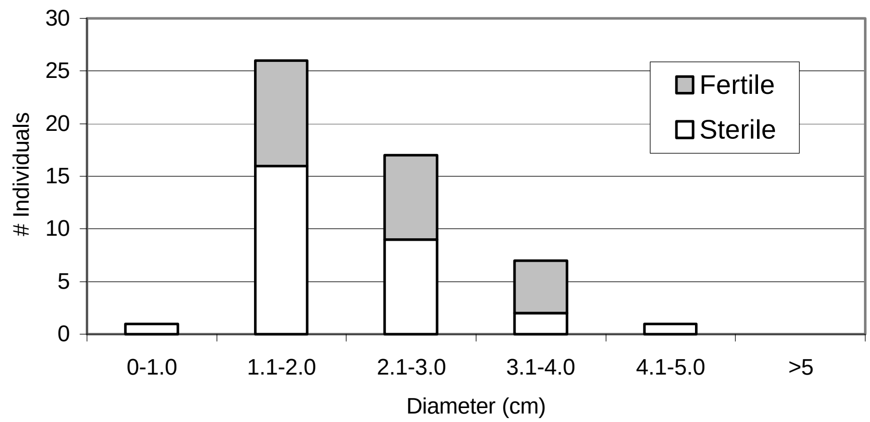

Figure 19. Distribution of basal diameter vs. reproductive status for all individuals of *Buxus vahlii* measured at the Rincón population during August 1997.

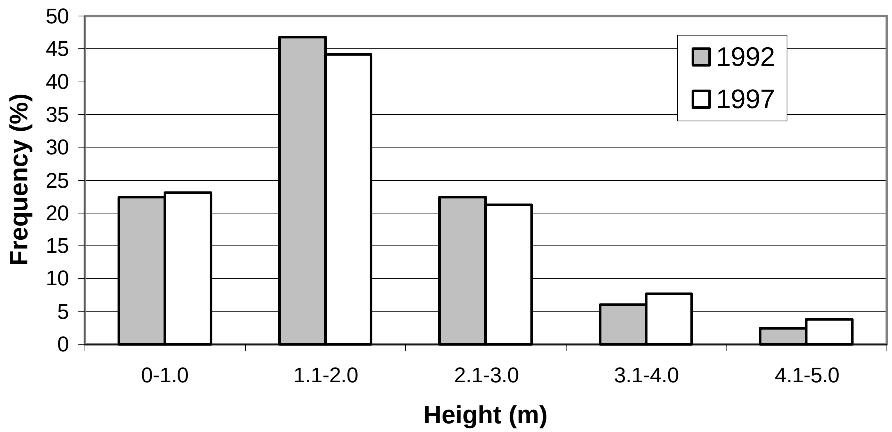

Figure 20. Comparison of the *Buxus vahlii* height distribution measured at the Rincón population during 1992 and 1997.

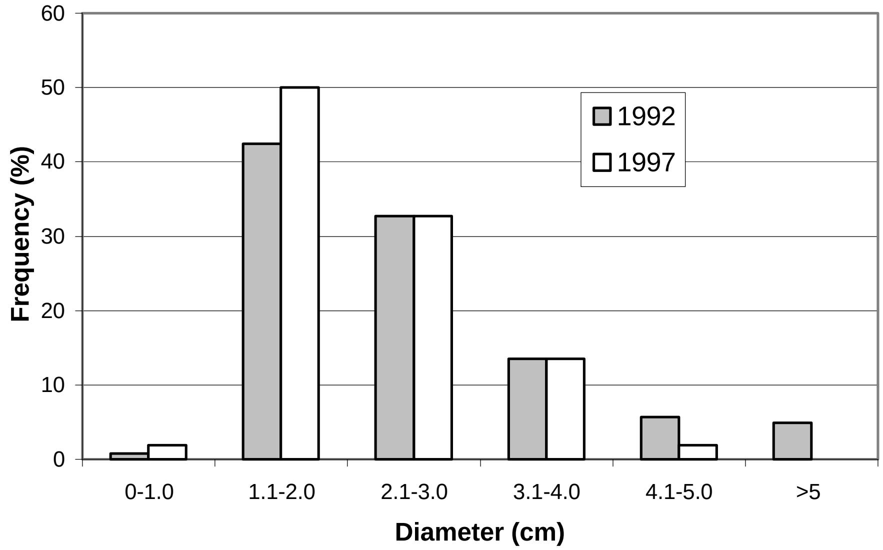

Figure 21. Comparison of the *Buxus vahlii* basal diameter distribution measured at the Rincón population during 1992 and 1997.

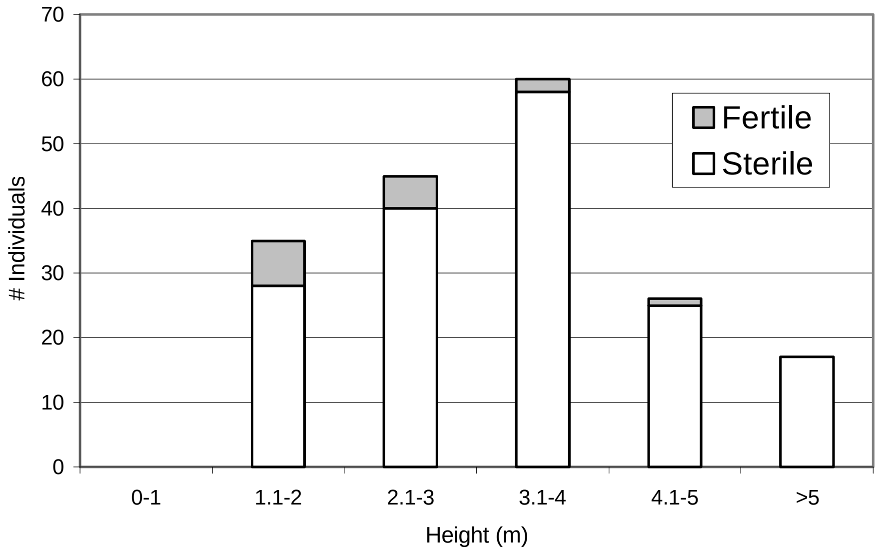

Figure 22. Distribution of height vs. reproductive status for all individuals of *Buxus vahlii* measured at the Isabela population during August and September 1997.

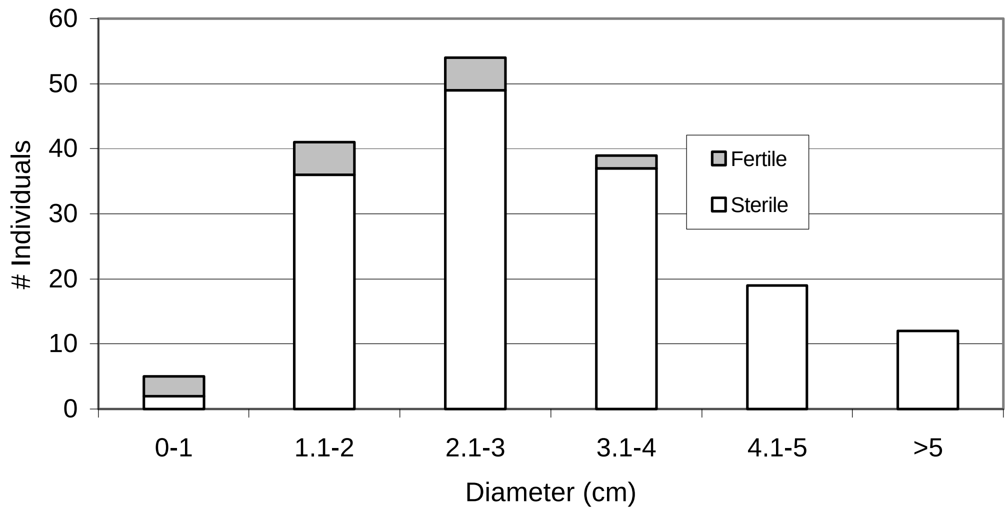

Figure 23. Distribution of basal diameter vs. reproductive status for all individuals of *Buxus vahlii* measured at the Isabela population during August and September 1997.

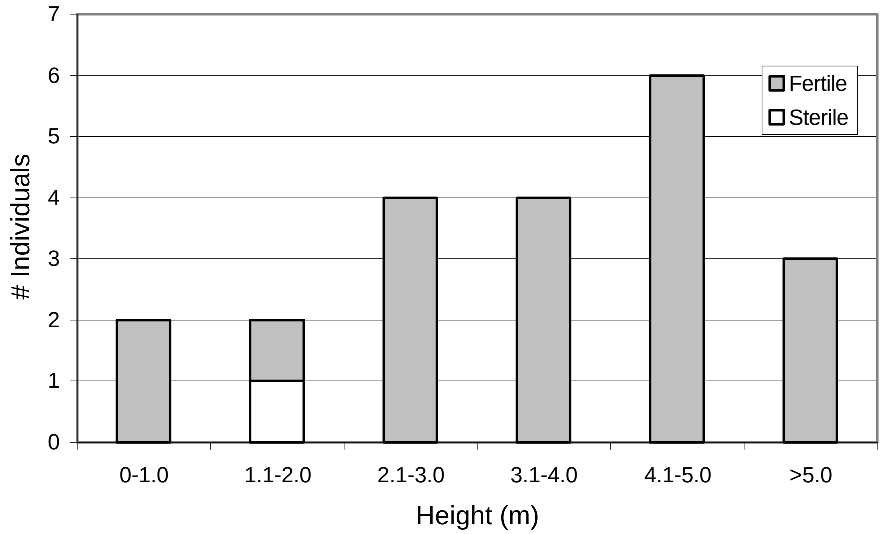

Figure 24. Distribution of height vs. reproductive status for all individuals of *Buxus vahlii* measured at Bayamón population during October 2000.

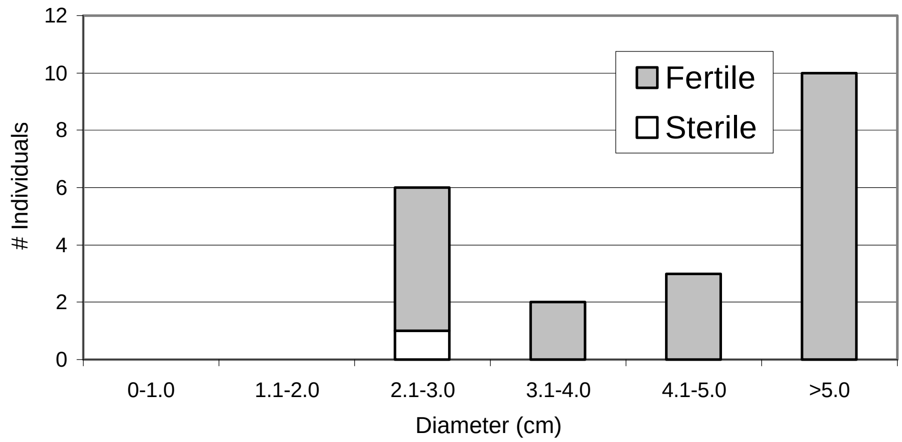

Figure 25. Distribution of the basal diameter vs. reproduction status for all individuals of *Buxus vahlii* measured at the Bayamón population during October 2000.