

**A STUDY OF *RANDIA PORTORICENSIS* (URB.)
BRITTON & STANDLEY [RUBIACEAE]: A RARE
SPECIES**

by

Javier A. Román Guzmán

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Approved by:

Lewis, Allen, PhD
Member, Graduate Committee

Date

Kolterman, Duane, PhD
Member, Graduate Committee

Date

Breckon, Gary, PhD
President, Graduate Committee

Date

Van Bloem, Skip, PhD
Representative of Graduate Studies

Date

Williams, Lucy, PhD
Chairperson of the Department

Date

ABSTRACT

Randia portoricensis (Rubiaceae) is an endemic shrub known historically from two locations in Guánica and Yauco. Six populations, including one found in 1992 at Montes de Barina in Yauco, with a total of 38 adults and 40 seedlings were located. The two populations on private land are threatened by cutting and development. Four small populations occurred in Guánica Forest Reserve. Between 1992 and 2005, 44 individuals in the Barina population were lost, which accounted for 54 % of the known plants. The species is dioecious with sphingid moths being the most likely pollinator. Fruits were found in five populations; two populations had seedlings. There was no evidence of fruit dispersal. *Randia portoricensis* meets three of the five possible IUCN criteria for listing as Critically Endangered. Propagation protocols and a strategy for the introduction of 50 juvenile plants to the four existing populations at Guánica Forest were developed.

RESUMEN

Randia portoricensis (Rubiaceae) es un arbusto endémico que históricamente se conoce en Guánica y Yauco. A través de este estudio se localizaron seis poblaciones con 38 individuos adultos y 40 plántulas, incluyendo una localizada en 1992 en Montes de Barina, Yauco. Las dos poblaciones que se encuentran en propiedad privada se encuentran amenazadas por desarrollo y poda. En el Bosque de Guánica se encuentran cuatro poblaciones pequeñas. Cuarenta y cuatro individuos han desaparecido en la población de Barina entre 1992 y 2004, lo que equivale a un 54 % de las plantas conocidas. La especie es dioica, siendo los esfinges los mejores candidatos como polinizadores. No hay evidencia alguna en cuanto a dispersores. *Randia portoricensis* cumple con tres de los cinco criterios de la IUCN para ser enlistada como en Peligro Crítico. Por último, se desarrollaron protocolos para la propagación y estrategias para la introducción de plantas juveniles en las cuatro poblaciones existentes del Bosque de Guánica.

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INTRODUCTION

Randia portoricensis (Urb.) Britton & Standley (Rubiaceae) is a rare shrub endemic to the dry forest in the southern part of Puerto Rico. The species is distinguished by its nearly sessile leaves (Fig. 1), hard, corky fruits (Fig. 2) and unusual spines (Fig. 3). Its spines are unique, at least among the West Indian species, in that they branch forming a trichotomy of three terminal spines (Britton and Wilson, 1925). The original description was based on fruiting plants, but by its placement in the genus *Basanacantha* the species is assumed to be dioecious. According to Liogier (1997:154) the flowers are unknown.

Little is known about the ecology, reproduction or distribution of *Randia portoricensis*. Historically the species was reported from coastal thickets around Ponce and in woods above Barina (Urban, 1964 [reprint] as *Basanacantha portoricensis* Urb.). Urban cited Sintenis 3744 and Sintenis 4880, both of which were collected in the 1870's. Britton and Wilson (1925) later give the distribution as Guánica and Ponce, with the former location apparently based on a fragment of Sintenis 3744 at NY, whose label reads "Barina near Guánica". Liogier more recently gave the distribution as the hills between Ponce and Guánica, which would include the municipality of Yauco (Liogier, 1997). In fact, no collections are known from the municipality of Guánica and the report of it from there apparently is based on a misunderstanding of the label on Sintenis 3744.

In 1994 Breckon and Kolterman reported on a population found by a graduate student (Rudy O'Reilly) in the dry limestone hills of Barrio Barina on private land that belonged to a Mr. Catalá in the municipality of Yauco. Dirt roads had been bulldozed in

the area in preparation for its development as an urbanization. The population had 51 individuals, of which 41 were adults and 10 were seedlings. Eight of the 41 individuals were observed in reproductive condition. No further studies have been done on *Randia portoricensis* since 1994 prior to the recent study, and nothing was known about the populations reported from Ponce and Guánica.

All of the historical and recent collections of *Randia portoricensis* are from the southern part of the island and on limestone substrate. The region falls in the Subtropical Dry Forest vegetation zone of Holdridge's vegetation classification system, which in Puerto Rico occupies an area of 1,216.4 km² (Ewel and Whitmore, 1973). The vegetation is characterized by low forest rarely exceeding 15 m in height with a mixture of evergreen sclerophyllous and non-sclerophyllous deciduous and semi-deciduous trees and shrubs that are often spiny, and the presence of cacti. The best example of Subtropical Dry Forest in Puerto Rico is the Guánica Forest Reserve, which has been protected for the last 88 years (Lugo et al., 1996).

The purposes of this study were to determine the status of the species, to obtain information about its reproductive biology and habitat requirements, and to propagate it for reintroduction.

REVIEW OF LITERATURE

Rubiaceae is perhaps the fourth largest family of flowering plants, with between 450-650 genera, and 6,500-13,000 species of herbs, shrubs, trees and lianas. Its diversity is greatest in the tropics with relatively few taxa in the temperate to boreal zones.

According to Anderson (1992), the Rubiaceae is represented by 4,555 species in the neotropics, and is divided in four subfamilies: *Cinchonoideae*, *Ixoroideae*, *Antirheoideae*, and *Rubioideae*. *Randia* Lam. is in the Tribe *Gardenieae* of the subfamily *Ixoroideae*.

Traditionally *Randia* was considered to contain between 200-300 species and to occur in both the Old and New Worlds (Liogier, 1997), but recent authorities are restricting the genus to the neotropics, reducing the number of species to 88, of which 12 occur in the West Indies (Anderson, 1992). More recently Gustafsson and Persson (2002) using a combination of molecular and morphological data concluded that the genus contains three groups: one in Mexico, Central America and the Antilles including subtropical Florida and the Bahamas, a second group in lowland South America and a third group of Andean species.

The neotropical species of *Randia* occur in deciduous and evergreen vegetation from between sea level and 3,300 m of elevation (Gustafsson and Persson, 2002). The genus can be distinguished from other members of the *Gardenieae* by the following characters: pollen in permanent tetrads, dioecious (female flowers with nonfunctional stamens, male flowers with a nonfunctional stigma and rudimentary ovary), thorns, conspicuous short-shoots with clustered stipules, unilocular ovaries with two parietal placentas and fruits with many discoid seeds embedded in a sweep pulp that turns dark

when dry (Gustafsson and Persson, 2002). There are exceptions to many of the above characters and the taxonomy of the genus has yet to be stabilized.

Three native species of *Randia* are reported in the Puerto Rican flora (Liogier, 1997). *Randia aculeata* L. is a widespread, highly variable species that according to Anderson (1992) is found in Central America, West Indies, northern Colombia, Venezuela, and Trinidad and Tobago. *Randia parvifolia* Lam. had been applied to a small-leaf form of *Randia aculeata* on Mona Island. Recent studies show that *Randia parvifolia* is restricted to Hispaniola and should be dropped from the Puerto Rican flora (Breckon, unpublished).

Rarity has been defined as the pattern of distribution and abundance of a species at a specific time, while endangered or threatened species as those more susceptible to decline or extinction (Falk et al., 1995). In 2003, Liu and Koptur attributed rareness and endangerment of species to intrinsic or extrinsic factors, or to anthropogenic activities. Kruckeberg and Rabinowitz (1985) expressed rarity as an interaction of three factors: size of the geographic range, habitat specificity, and local population size, thus making rare species more susceptible to threats than common species (Falk et al., 1995). Based on their criteria *Randia portoricensis* is a rare species, in fact, according to Clewell (1985), its limited range and numbers place it in the category of very rare.

Biologists reintroduce rare plants to appropriate habitat and historic range for a variety of reasons and reintroduction may play an important role in the recovery of rare species (Pavlik, 1995). In Hawaii, where almost one-third of the flora is either extinct or in danger of extinction, the reintroduction of rare species has been a very useful practice

(Mehrhoff, 1993). There are protocols that need to be followed for successful reintroduction (Falk et al., 1995). These protocols can be divided into technical, ecological, strategic and political categories. Technical problems involve choice of species and site, method of reintroduction, e.g., seed, seedlings, saplings or mature plants, timing for introduction, post-introduction treatments and monitoring. Ecological considerations include number of individuals needed to obtain a sustainable population, which will vary with the species, depending on genetic variation, breeding system and pollination and seed dispersal requirements (Falk et al., 1995).

The strategic and political considerations are dependent on who owns the land, what the future plans for the lands are, and who is going to guarantee the monitoring phase. Finally, the criteria used to measure success must be defined specifically for the taxon being treated, as success is an abstract or relative concept that includes measures of abundance, extent, resilience and persistence. In order to get a successful management and recovery program a good knowledge of pollination biology and breeding systems of rare and endangered species is needed (Liu and Koptur, 2003).

It is estimated that the native vascular flora of Puerto Rico consists of about 3,000 species (Liogier and Martorell, 2000), of which 48 or 1.6 % of the flora are listed by the U. S. Fish and Wildlife Service as endangered (<http://www.fws.gov/>). The majority of these species occur either in the northern Karst region or in the dry south-southwest portion of the island (<http://www.fws.gov/>). Breckon (unpublished) estimates that between 30 % and 40 % of the flora are composed of non-native species whose presence on the island are due to human activities. If this estimate is correct, then it would

increase the proportion of endangered species to 2.3-2.7 % of the native flora. Recent studies by Breckon and Kolterman (unpublished data) indicate that there are a number of plant species that should be federally listed as endangered and that more than 60 species previously reported from the island have a high probability of being either extinct or extirpated.

Puerto Rico was nearly 100 % forested when Europeans arrived in 1493. By 1940 over 90 % of the island's forests had been cleared, primarily for agriculture, fuel and timber and only 1% of the forest could be considered as undisturbed primary forest (Lugo, 2004). With the passage of the Industrial Incentive law in 1947, a shift from agriculture to manufacturing occurred and large segments of the population migrated to urban areas, which resulted in a change in land usage. While the period between 1950 and 1990 resulted in proportionally the largest event of forest recovery anywhere in the world, the majority of the forests were dominated by exotic species (Lugo, 2004). Further, with the increase in population and the growth in urbanization, the nature of the disturbance changed from cutting and burning for agriculture and fuel to the more destructive bulldozing and clearing for roads, transmission lines, ports, refineries, mines, power plants, urbanizations, shopping malls and industrial centers.

The response of the flora to the clearing of the vegetation, the introduction of exotic species, and the recovery of the forests with the setting aside of preserves and reserves has not been uniform. One effect of habitat fragmentation is the increase of edge habitats (Liu and Koptur, 2003) and another effect is the decrease in large, intact areas of native vegetation. Some of the native species have expanded their numbers while others

have suffered losses and are seriously threatened with extirpation or extinction or have already been lost from the island (Breckon, Kolterman and Vélez, unpublished data). Scattered studies of individual species document a loss of populations and individuals in a number of cases: e.g., *Polygala cowellii* (Britton) Blake (Rojas, 1994), *Goetzea elegans* (Urb.) Urb. (Santiago, 1996), *Sabal causiarum* (O. F. Cook) Becc. (Breckon and Kolterman, 2000), *Gesneria pauciflora* Urb. (Breckon and Kolterman, 1994a), *Harrisia portoricensis* Britton (Santiago-Vélez, 2000), *Myrciaria myrtifolia* Alain (Breckon and Kolterman, 2003). A systematic survey is underway by Breckon, Kolterman, and Vélez to document changes in the flora as a whole, but it will be years before the work is completed.

Habitat fragmentation has been a dominant process in landscapes over the last century and today it is a major threat to rare and endangered species. Recently, Holl (2002) attributed the lack of studies in tropical forest succession to the growing area of abandoned agricultural lands. The IUCN (2002) reported that habitat loss and degradation threatens 91% of the rare plants in the world. In Puerto Rico habitat fragmentation has occurred extensively over the past 500 years (Lugo, 2004). For example, before its being set aside as a Reserve, the Guánica Dry Forest suffered extensive cutting for charcoal and fence-post production and clearing for agricultural crop production (Lugo et al., 1996; Ramjohn, 2004). In the Sierra Bermeja in southwestern Puerto Rico, the La Tinaja tract of the U. S. National Wildlife Refuge was cut for timber during the 20th century and today is covered by secondary dry forest (Weaver and Chinea, 2003).

Negative impacts of habitat fragmentation on populations include a loss of genetic variability, bottleneck effects, genetic drift (Young and Brown, 1999; Murren, 2002; Donath et al., 2003), a loss of potential breeding partners (Rojas, 1994), reduction or loss of pollinator (Lienert and Fischer, 2003) and seed dispersers (Donath et al., 2003; Wehncke et al., 2003), and a loss of potential habitat for seedling germination (Rojas, 1994; Young and Brown, 1999; Murren, 2002). Finally, a decrease in population size increases the probability of chance extinction (Young and Brown, 1999). The reintroduction of individuals into small populations with lower fitness may restore fitness to levels comparable to those before the effects of the genetic drift (Hedrick and Kalinowski, 2000).

While habitat fragmentation usually has negative effects on plant populations, especially on rare and endangered species, recent studies have shown evidence that for some species habitat fragmentation may be positive. Possible positive responses to habitat fragmentation include local adaptation after isolation, an increase in population size with an increase in habitat edges (Cunningham, 2000), and continuing gene flow between populations (Murren, 2002).

Along with habitat fragmentation other major causes of extirpation and extinction are introduction of exotic species resulting in competition, predation, or hybridization, over-harvesting and pollution (Holl, 2002; Clark and Wilson, 2003). Allen (2000) reported that 90% of the Hawaii's dry forest has been eliminated due to the introduction of fires, alien grasses, cattle ranching, and feral ungulates.

Reproductive ecology

The life cycle of a plant can be broken down into eight phases: vegetative, flowering, pollination (including breeding system), fertilization, seed production, seed dispersal, germination, seedling establishment, and back to vegetative growth. Little is known about what stage is more important to plant growth and reproduction (Ellison and Parker, 2002). Pollination and dispersal success can be assessed if we can evaluate consecutive losses of new individuals at different stages during their life cycle (Traveset et al., 2003). Although we can link some of these stages together, i.e. that of pollination and seedling recruitment (Traveset et al., 2003), it is difficult to say which stage is more important. Ellison and Parker (2002), Holl (2002), Clark and Wilson (2003) and Castro et al. (2004) attribute the failure to reproduce or mortality at either the individual or population level to the disruption or failure of seed dispersal and germination, and seedling establishment. Apparently there is no literature available that indicates how to determine the most critical phase or phases, but according to many studies (Ellison and Parker, 2002; Holl, 2002; Clark and Wilson, 2003; Castro et al. 2004) one can suggest that observations, measurements and experimental manipulation are necessary to determine which phase or phases are responsible for the failure.

Flowering

Randia portoricensis was originally described in the genus *Basanacantha*, which was characterized in part by being dioecious. *Randia (sensu lato)* is treated to include species with both bisexual flowers and unisexual flowers (Liogier, 1997) with the taxa

having unisexual flowers being dioecious. Baker (1967) argued that dioecy would be more common in island plants compared to continental species due to selective pressure for outbreeding in a flora where the ancestral species were self-compatible. Bawa (1980) estimated that 3%-4% of all angiosperms are dioecious and that the Hawaiian Islands and New Zealand have floras richer in dioecious species compared to continental floras. He found that even the flora on the recently formed Barro Colorado Island, in Lake Gatún in Panama, had twice the number of dioecious species compared to the forests surrounding the lake (Bawa, 1980). More recently Renner and Ricklefs (1995) raised the estimate of dioecy of all angiosperms to close to 6% (14,620 out of 240,000 species). However, the occurrence of cryptic dioecy, where species appear to have hermaphrodite flowers, but the flowers on different individuals function either as male or as female (Naiki and Kato, 1999; Kawagoe and Suzuki, 2004), could result in an underestimation of the frequency of dioecy. Flores and Schemske (1984) using descriptions from floristic treatments found that 125 species out of 2,037 (6 %) in the Puerto Rican flora were dioecious, which agrees closely with Renner and Ricklefs' estimate for angiosperms in general.

While dioecy promotes outcrossing, it does so at a price. Assuming that in a population of a dioecious species the number of male and females are the same, "only half of the parent plants contribute to seed dispersal" (Nanami et al., 1999). Nicotra (1999) argued that in dioecious species, males can achieve greater growth, higher frequencies of reproduction and can flower at lower light availability than females. In *Siparuna graniflora* (Kunth) A.DC., a neotropical dioecious shrub, females allocate more biomass to reproduction than males (Nicotra, 1999). Negrón (1987) showed that

female plants of *Zamia* coned less frequently than male plants, which she assumed was due to the greater biomass and longer development period of the seed cones compared to the pollen cones.

Related to dioecy is the occurrence of heterostyly, where different flower morphs occur in the population. The classic distylic condition is for flowers on half the individual plants to have long styles and short stamens (pin flowers) while the flowers on the other half of the population have short styles and long stamens (thrum flowers) (Percival, 1965). There are numerous variations from the classic condition (see Percival for a discussion). The Rubiaceae contains many dioecious species (Naiki and Kato, 1999) which are often distylic (Cronquist, 1981; Naiki and Kato, 1999). Ornduff (1966) gave evidence that the distylous condition involved a super-gene that consisted of a number of contiguous loci that were inherited as a unit. The loci coded for various features including style length, filament length, pollen size, and breeding system (if self-incompatible).

Pollination

Prior to this study, the flowers of *Randia portoricensis* were unknown, thus making it difficult to determine what the pollination requirements are. However, based on the other species in the genus and for that matter the family, it is reasonable to assume that the flowers are zoophilous. Recently it has been stated that 90 % of the angiosperms are pollinated by animals (Engel and Irwin 2003) and those of the Rubiaceae are “mainly entomophilous, only rarely anemophilous” (Cronquist, 1981). The flowers of *Randia* are

characterized as bisexual or unisexual, usually white, or sometimes yellowish, with a campanulate, funnelform or salverform corolla (Liogier, 1997), which strongly indicates zoophily (Bawa, 1980). Further, there is evidence for the need of animal-mediated pollination in the Puerto Rican flora. For example, *Polygala cowellii* requires the carpenter bee (*Xylocopa mordax*) for pollination (Rojas, 1994) and *Goetzea elegans* requires visitation by either the Bananaquit (*Coereba flaveola* Bryant) or the Antillean Mango hummingbird (*Athracothorax viridis* Audebert & Vieillot) (Santiago, 1996). Both plant species are trees endemic to Puerto Rico.

Relatively few pollination and breeding system studies have been done on woody plants in Puerto Rico and even fewer on rare endemic species. Rojas (1994) found *Polygala cowellii* to be an obligate outcrosser with two exceptions where isolated individuals did set seed. However, fruit production, seed germination, seedling survivorship and growth were significantly lower in the two selfing individuals than in closely spaced (assumed outcrossing) trees. Santiago's findings (Santiago, 1996) suggest that *Goetzea elegans* is self-compatible and showed no signs of inbreeding depression. *Piper blatterum*, an endemic understory shrub, either is autopollinated or apomictic (Reyes, 1993). Six species of Melastomataceae varied from apomictic to autopollinated to zoophilous. The latter varied from having a secondary autopollination system to being obligate outcrossers (Dent, 1989). Breeding system and pollination system showed a strong correlation with geographic range, with widespread species being either apomictic or autopollinated and zoophilous species being endemic (Dent, 1989).

Because of the common occurrence of self-incompatibility in dioecious plants (Bawa, 1980), inbreeding in small isolated populations can be very pronounced and can reduce their genetic variability (Lienert and Fisher, 2003). Inbreeding depression may result and then population viability and individual fitness can be lower (Lienert and Fisher, 2003). If *Randia portoricensis* is pollinated by species characterized by moving over relatively long distances between flowers and plants then possible problems of inbreeding or pollination failure may be mitigated (Murren, 2002).

It has generally been accepted that rare plants, largely because of limited geographic ranges, will have lower genetic variability than widespread species (Silva-Montellano and Eguiarte, 2003). Further, genetic drift and loss of generic variation are highly marked in small populations (Cole, 2003). However, when widespread and rare congeneric species are compared, the difference in genetic variability is not as strong as when rare species are treated as a group without phylogenetic consideration (Gitzendanner and Soltis, 2000). Even so, after measuring the genetic variability of 57 genera, rare species showed a reduction compared to their common congeners (Cole, 2003).

How to apply either the general rarity model or the congener findings to *Randia portoricensis* is unclear. Only a few of the 34 species pairs used by Gitzendanner and Soltis (2000) were woody or tropical. The majority of the species were temperate zone herbs and Silva-Montellano and Eguiarte (2003) have shown that correlations exist between growth habit and genetic variability. Further, the present day rarity of *Randia portoricensis* may not reflect its past distribution and abundance, but rather it may be due

to the extensive clearing of the vegetation that has occurred over the past 500 years.

With the current knowledge, it is not possible to determine if the genetic variability in *Randia portoricensis* has been affected by human activities. Finally, morphologically there is no related species to *Randia portoricensis* in the Caribbean, at least based on fruit characters, that allows for testing it against closely related congener species. As best as can be determined from the literature and herbarium material, all of the other Caribbean species of *Randia* have smaller, fleshy berries. None have a corky phase in their development.

Seed production and dispersal

Randia portoricensis produces 24-40 seeds per fruit (Breckon and Kolterman, 1994), but up to now data have been available as to seed or fruit production at the individual or population level or as to the number of fruiting events per year. This lack of data is critical as seed production is an essential stage in life cycle of plants. Seeds are the unit for dispersal in most species and they play an important role in the colonization of new habitats (Wehncke et al., 2003). Seed production can be affected by individual plant size, age, robustness (Kimura et al., 2002; Negrón, 1987), population structure (i.e., see previous discussion of pollination), habitat and predation (Tomimatsu & Ohara, 2002; Jump and Woodward, 2003; Liu and Koptur, 2003).

Habitat modification can affect seed production. Liu and Koptur (2003) found reduced seed output due to increased seed predation in *Chamaecrista keyensis* Pennell individuals adjacent to urban areas compared to plants in pristine forest. They found that

seed predation by insects could cause seed or fruit abortion or even both and affected seed germination.

Dispersal can be divided in two stages; travel and establishment. Dispersed the seeds then become the starting point for subsequent recruitment stages (Castro et al., 2004). Fruit and seed morphology, color and odors can provide clues as to possible dispersal mechanisms (van der Pijl, 1979; McMurray et al., 1997; Nakanishi, 2002; Levine et al., 2003; Wehncke et al., 2003; How and Miriti, 2004). The dispersal mechanism for *Randia portoricensis* is unknown (Breckon and Kolterman, 1994), and with its unique hard, corky fruits does not fit well into any of the various dispersal syndromes. No reports in the literature have been found about dispersal in corky fruits.

Both seed dispersal and seed and seedling predation can have a strong effect on population distribution (Radford et al., 2002). In part, a species' distribution depends on its ability to disperse its seeds (Nanami et al., 1999) and on the distance and direction in which the seeds are dispersed (Levine and Murrell, 2003). In the seed stage offspring have a greater chance to escape the parent plant and to colonize new and potentially more favorable habitats (Wehncke et al., 2003) as well as to decrease extinction rates (Clark & Wilson, 2003). After dispersal has occurred, seeds can land on the soil surface where they can become buried and enter the seed bank, or germinate, or die as a consequence of competition, disease, predation, senescence, and abiotic factors (Clark & Wilson, 2003).

Post-dispersal seed predation can contribute greatly to seed mortality in many ecosystems, thus limiting the chances of new seedlings to establish in new habitats (Janzen, 1971; Ibarra-Manríquez et al., 2001). However, dispersed seeds are more likely

to survive, germinate, and establish by escaping predation, pathogens, herbivores (Wehncke et al., 2003) and intraspecific competition than non-dispersed seeds (Benítez-Malvido et al., 2003). In 53 species studied, predation and competition had a negative effect on seedling recruitment (Wehncke et al., 2003). For species with small populations that do not have successful dispersal, seed predation or damage can be highly destructive (Mull and MacMahon, 1997; Holl, 2002).

Seed predation can be more destructive (Benítez-Malvido et al., 2003) than seed damage, because partially damaged seeds may develop and germinate if the embryo is still alive after the damage (Koptur, 1998). Species whose seeds have reduced reserves due to predation damage or whose numbers are decreased due to predation may not be as good competitors as species which start with greater numbers of intact seeds (Koptur, 1998). There are no reports of seed predation in *Randia portoricensis*, so it is not possible to determine if seed predation has played a role in its population dynamics.

Germination

Temperature, light requirements, and moisture are critical in germination (Dai et al., 2002; Radford et al., 2002; Bakar and Nabi, 2003; Zia and Ajmal, 2004). For example, Zia and Ajmal (2004) showed that soil conditions, e.g. salinity, and moisture influence the timing of germination of 27 seed species identified during their study between 1996 and 1997. Of these 27 species 12 established during the first year (1996) and the following year 15 species were found. Dai et al. (2002) found that seedling survival between two growing seasons was significantly different due to topographic

position and light conditions. Radford et al. (2002) infer that high seedling mortality may be due to droughts during the year of the study. In 2003 Bakar and Nabi manipulated seeds in laboratory to determine whether the temperature, light conditions or chemical media influence seed germination. They found that temperature and light conditions influence seed germination in wrinklegrass (*Ischaemum rugosum* Salisb.).

In Puerto Rico, seed germination in dry forest tree species is promoted by sufficient amounts of moisture in the areas where seedlings established, a factor controlled by rainfall patterns (Carvajal, 2002). Assuming that this moisture is not available, seed dormancy could become adaptive (Carvajal, 2002).

The only collection known of *Randia portoricensis* with reproductive material in Puerto Rico is at the MAPR Herbarium (UPR-RUM), and the only propagation study for the species was published by Breckon and Kolterman (1994b) in collaboration with USFWS. In this paper, they reported that seeds germinated in 9-30 days; 9-11 days for shady conditions and 25-30 days for light. This is the only propagation attempt for the species, and no further studies were made since 1994.

Establishment

It is more difficult to apply existing models of dispersal to plants in isolated habitats (Ellison and Parker, 2002). Ellison and Parker (2002) argue that some of the models implied long-distance dispersal, but for many habitats this is not the case because with the increase in urbanization and other landscape transformations neither establishment nor the stopover of dispersers will occur. Unique soils, habitats, and

restricted distributions make many of the world's rare plant species edaphic endemics and vulnerable to human activities (Maschinski et al., 2004).

Dispersal and establishment are essential during the plant cycle, but less is still known about them than earlier phases (Ellison & Parker, 2002). For every species seedling establishment is an essential step for population regeneration, but for some species this stage is critical because it represents high mortality rates and for the potential to change spatial and temporal patterns of recruitment (Castro, et al., 2004). Ibarra-Manríquez, et al. (2001) found that seedlings adapted to shady understory conditions have higher survival under low light intensities compared to high light conditions.

Thus, it can be summarized that after seeds are dispersed, seedling establishment is critical for both the distribution and regeneration of the species. Dai et al. (2002) attribute seedling dynamics and establishment to factors like soil moisture, habitat disturbances including canopy losses and recoveries, and leaf litter layers. Noe and Zedler (2001) studied the spatio-temporal variation of seedling establishment and found, contrary to their expectations, that soil salinity and moisture explained little of the spatio-temporal variation in seedling establishment, but did explain timing of germination.

After studying causes of seed dispersal failure, Castro et al. (2004) found that death of seeds and seedlings was the primary fate for three of the four boreal tree species studied. The causes of death were summer drought (69-74 %), trampling (22-29 %), and herbivory (1-2 %). Although their study focused on tree species, they found shrubs showed a higher survival rate. How these results would translate to the subtropics of Puerto Rico is not clear.

METHODS

Distribution

Location data were obtained from interviews with plant taxonomists, the literature, and consulting herbaria in Puerto Rico and the United States having major holdings of Puerto Rican collections (MAPR, NY, SJ, UPR, UPRRP, US). Where possible these locations as well as those found in the present study were mapped on USGS 7.5 minute topographic maps. Locations, as determined by GPS (Garmin *etrex*; 15 m circle of accuracy) and by topographic features on the maps were compared for accuracy.

Population

Between August 2002 and May 2005 all four of the reported locations were visited as well as four of the probable locations. Each area was carefully searched for the species. All individuals, except seedlings were tagged, measured (height, dbh, number of stems from the base) and reproductive status recorded (sterile, flowers, fruits). Individuals 0.5 m high or higher were assumed to be large enough to flower. Height, rather than stem diameter was used due to the slender nature of the stems, the difficulty in getting diameter measurements due to the spines, and the fact that many of the plants had two to many slender stems from the base. Reproductive individuals as small as 0.5 m high were found. Seedlings were based on both size of the individual (< 10 cm high); in one population there were no seedlings present the first year, but they were present the second year, so that size and age in this case was correlated. One seedling was dug from the ground to be sure that they were seedlings and not root suckers. Distances and

compass directions between individuals were measured for each population. GPS readings were obtained for each individual and population. Habitat conditions (slope, aspect, light conditions, associated species) were recorded for each population. USGS 1:20,000 series maps were used to determine elevation, topographical features, and place names. Vegetation was determined by direct observation and by consulting Ewel and Whitmore's (1973) map of Holdridge's Ecological Life Zones for Puerto Rico and the Virgin Islands. Geological formation and the soil type were determined using USGS I-1147 and I-1042 maps (Krushensky and Monroe, 1978a, 1978b).

Application of the IUCN criteria

The extent of occurrence was determined by mapping the location of all populations and drawing a minimum convex polygon to include all the points. No attempt was made to exclude discontinuities or disjunctions within the area. Major discontinuities would be Puerto de Guayanilla, Bahía de Guayanilla, and Bahía de Tallaboa. The extent of occurrence was determined for the species and for each population. The area of occupancy was arbitrarily set as an estimated area occupied by an individual based on its projection on the ground under the tree. This varied greatly, but appeared to be primarily a function of the number of primary stems from the base of the plant. Seedlings were not included in the determination as they were never encountered outside the projected crown area under the parent plant. Large plants would have a crown 2 to 2.5 m in diameter. I used a square 3 m per side, which was an overestimate for the majority of the individuals,

as the per-individual area of occupancy and summed these to obtain the area of occupancy of the population.

Germination tests

Germination test 1. Green fruits were collected from one individual (# 244) in the Barina population in November 2002. The fruits were stored in paper bags in the laboratory for one month in an attempt to ripen them. Seeds were extracted by cutting open the fruits and using forceps to separate individual seeds, which were measured using calipers and weighed. To reduce the possibility of pathogens, the seeds were then soaked in a 10 % chlorine bleach solution (5 ml of Clorox® and 45 ml of distilled water) for 10 minutes and then rinsed in distilled water. The treated seeds were placed on moist paper towels in Petri dishes (5 per dish) for germination and maintained in the lab. Water was added as needed to maintain a moist environment. The seeds were checked once a day for germination over a 10-week period.

Germination test 2. Between late February and mid-March 2003 fruits that were gray were collected from the only two fruiting trees of the Barina population (# 244, 247) and stored for eight weeks in paper bags in the lab at room temperature until they turned bluish-black. The collected fruits, seeds and resulting seedlings were marked as to parent plant. Treatment of the fruits and seeds was similar to the first treatments except that the seeds were planted in a commercial soil mix in 3” pots. The pots were watered daily and germination and growth data were taken weekly. Periodically, as needed, all the seedlings or juvenile plants were transplanted to larger pots and kept in the greenhouse.

Germination test 3. Between late April and mid-May 2004, bluish-black fruits were collected from the only fruiting individual (# 247) of the Barina population. The fruits were soaked in distilled water for two days in the lab to facilitate their opening; the fruits were then broken open by hand and the seeds removed. The seeds were planted in commercial soil mix in 5" pots and maintained in the greenhouse. Commercial 20-20-20 fertilizer was applied periodically as needed. In October, the seedlings, which were between 0.5 - 6.0 cm in height, were transplanted into larger pots. Originally, half of the seeds were placed in full sun and the other half were placed under 70 % shade cloth to test for the effect of light on germination, growth and survivorship. After 30 days someone moved all of the pots to the full sun, mixing the two groups in the process.

Seedling growth and survivorship

Survivorship of greenhouse seedlings was followed for 11 (July 2004 planting) or 13 months (May 2003 planting). Seedlings in the Barinas population were counted and marked in May 2004 and recounted on 30 September 2005. To determine if they were individual seedlings or root suckers, litter layers were removed for a detailed observation. One seedling was removed from the wild to determine if they were germinating in clumps. Seedlings in the greenhouse and those found in nature in the Barinas population were measured for growth. The greenhouse seedlings were approximately 18 months old (May 2004); it is assumed, based on size and the timing of their appearance that the wild seedlings were of a similar age. Plant height above the ground, number of leaves and number of branches were recorded.

Flowering and pollination

Field studies were conducted between August 2002 and December 2005 at the Barina population. During the late spring and summer of 2004, 50 flowers of *Randia portoricensis* were observed and collected from individuals # 244, 247, 260. The flowers were collected directly from the individual branch when possible; in some cases they were taken from the ground under a flowering individual. Collected flowers were fixed in FAA and transferred to 70 % ETOH for dissection. Each bottle was labeled as to the number of the individual plant, date and time of collection, and where collected (branch or ground).

In the lab the flowers were measured and dissected and photographed. Anthers, pollen, stigmas and dissected ovaries were examined using Nomarsky and fluorescence microscopy.

During the spring and summer 2005, individual flowers were marked on cultivated and wild plants and followed from anthesis till abscission. Observations were made every four hours starting at 7 a.m. and continuing until 3 a.m. the following morning (7 a.m., 11 a.m., 3 p.m., 7 p.m., 11 p.m. and 3 a.m.). Between five and 15 flowers were observed each day over a period of six weeks. Data taken were time of opening, functional time span of the flower, timing of scent production. The presence of nectar was determined by gently squeezing the corolla tube and observing if liquid became visible at the throat.

Observations for pollination were made during the spring and summer of 2004.

Observations were made at night between 5 p.m. and 7 a.m. for a continuous two week period.

Fruit and seedling dispersal was measured by counting the number of each within three concentric 0.5 m wide rings beginning at the base of the tree. Counting was stopped at 1.5 m as no fruits and or seedlings were found beyond that distance. Fruits and seedlings were counted only under individual # 244 and fruits under individual # 247. These were the only plants found with fruits or seedlings under them.

RESULTS

Individuals of *Randia portoricensis* were found at six of the nine locations visited (Fig. 4). The number of individuals varied from one to 25 in the six populations (Table 1). No individuals were found at the Cerro Presidio in Coamo, at the Punta Vaquero area in the Guánica Forest Reserve, or at the Cueva Convento area in Guayanilla. The latter three areas were searched as possible habitats for the species.

Population 1: Barinas, Yauco

The population is located on private property in the low limestone hills of the Montes de Barinas east of the small urbanized area of Limas in the Barrio Barinas of the Municipality of Yauco (Fig. 5). Limas is south of Cambalache on route 359. The population is reached by a dirt road that starts at the end of the paved road and houses in Limas.

Twenty-five assumed adult individuals were counted and tagged between 2002 and 2003 (Table 1). Two of the 25 individuals were tagged during the 1992 census; the other 23 were previously untagged. In September 2005 only 20 individuals remained. Cutting for fence posts occurred in the area in May 2005 and it is assumed that the five missing individuals were cut at that time. The extent of occurrence for the population formed a roughly triangular area of approximately 50,400 m² (estimated from map) with the two extreme individuals about 450 m apart. The plants were clustered in groups of four to six with several isolated, outlying individuals (Fig. 6). Two of these were approximately 70 m apart and about the same distance to the nearest cluster of

individuals. The most isolated individual was 279 m from its nearest neighbor (Fig. 6). The majority of the individuals were 15 m or less from their neighbor. The area of occupancy for the population in 1992 was estimated to be 96 m², which at the last census made in May of 2005 had dropped to 80 m². The Barina population was closest to the Trichilia Canyon population (4.2 km, map distance).

The plants ranged from 0.3 to 5 m in height with a median height of 1 m. Sixteen of the plants were less than 2 m tall (Table 1). Thirty-seven seedlings were found. Ten of the 25 adult individuals were found with either flowers or fruits or both over three reproductive seasons. Of the ten fertile plants five were known females (identified by fruits), one was a known male (identified by flower dissection), and four were seen to flower but their sex was not determined. Fruit production was dominated by plants # 244 and 247, with each plant producing more than 40 fruits in each of the three fruiting seasons. Plants # 204, 249, 251 and 256 produced only a single fruit each during the three years of observation. The only known male plant was number 260, which flowered all three years.

The site has the following characteristics:

Substrate: Oligocene limestone, but very close to and perhaps also occurring on Miocene Ponce limestone. The contact between the two formations is inferred from the map (USGS I-1147).

Coordinates: 18° 01' 03.1" N, 66° 49' 44.5" W

Elevation: 110 to 130 m

Aspect: On a gradual, continuous north-facing slope.

Light conditions: Open shade under thin canopy cover.

Vegetation: Subtropical Dry Forest, with evidence of disturbance (bulldozed dirt roads, cutting for fence posts, hurricane breakage).

Associated species: *Acacia farnesinana* (L.) Willd. (Mimosaceae), *Bourreria succulenta* Jacq. (Boraginaceae), *Bucida buceras* L. (Combretaceae), *Bursera simaruba* (L.) Sarg. (Burseraceae), *Coccoloba diversifolia* Jacq. (Polygonaceae), *Comocladia dodonea* (L.) Urb. (Anacardiaceae), *Erythroxylum rotundifolium* Luman (Erythroxylaceae), *Guaiacum sanctum* L. (Zygophyllaceae), *Leucaena leucocephala* (Lam.) de Wit (Mimosaceae), *Machaonia portoricensis* Baillon (Rubiaceae), *Pictetia aculeata* (Vahl) Urb. (Fabaceae), *Pilosocereus royenii* (L.) Byles & G. Rowley (Cactaceae), *Prosopis juliflora* (Sw.) DC. (Mimosaceae), *Randia aculeata* L., *Tamarindus indica* L. (Caesalpiniaceae), *Trichilia triacantha* Urb. (Meliaceae), *Vanilla barbellata* Reich. f. (Orchidaceae), *Zamia portoricensis* Urb. (Zamiaceae).

Population 2: Peñón de Ponce. Encarnación, Peñuelas

The population is located on private property in an east-west oriented canyon west of the correction facility Las Cucharas on the north side of Highway 2 (Fig. 7). The population can be reached by entering at the Texaco gas station west of the entrance to a gated community, and walking northeast to the small canyon. The population was located near the north end of the canyon.

The population consisted of four individuals in an estimated area of 10 m² (Table 1). A single GPS reading was used for the group. The estimated area was taken in this case as both the extent of occurrence and the area of occupancy. It was the most isolated of the populations, being 15.2 km (map distance) from the Barina population. One individual had fruits in the 2003 flowering season (fruit observed and collected in January 2004). Flowering was never observed. No seedlings were found and no evidence of old fruits was found around the plants.

Site characterization for this population:

Substrate: Miocene Ponce limestone (USGS I-1042)

Coordinates: 17° 59' 12.6" N, 66° 42' 10.8" W

Elevation: 50 m

Aspect: In bottom a canyon with dry river bed that runs north-south.

Light conditions: Shade, in understory.

Vegetation: Subtropical Dry Forest, with evidence of disturbance (bulldozed dirt road for an old trash dump; hurricane breakage; subdivision on adjacent hillside, gas station at mouth of canyon).

Associated species: *Coccoloba diversifolia* (Polygonaceae), *Eugenia woodburyana* Alain (Myrtaceae), *Randia aculeata* (Rubiaceae), *Trichilia triacantha* (Meliaceae), *Thrinax morrisii* H.Wendland (Arecaceae), *Zamia portoricensis* (Zamiaceae).

Populations 3 – 6: Guánica Forest Reserve

Population 3. Cañón de los Murciélagos, Guánica, Bo. Carenero/Yauco, Bo. Barina boundary (Table 1; Fig. 8)

The population is located in the “Cañón de los Murciélagos”, which is south of and beyond the southern end of the Cañón de los Murciélagos Trail. That trail branches west off of the Dinamita Trail. It begins as a dirt road but about midway it becomes a poorly defined trail that eventually reaches the canyon.

The population consisted of a single relatively large individual (#389) 5 m in height (Table 1). The extent of occurrence and area of occupancy of the population was 2 m². The nearest neighbor is the Caña Gorda population which is 1.32 km (map distance) away. One fruit was observed on the plant in October 2005. No evidence was found of other plants or seedlings or old fruits in the area.

Site characterization for this site:

Substrate: Oligocene limestone (USGS I-1147). Rocky soil with dead trees on the ground.

Coordinates: 17° 57' 58.0" N, 66° 51' 46.9" W

Elevation: 70 m

Aspect: In bottom at the north end of a north-south oriented canyon.

Light conditions: Open shade of understory with thin canopy cover.

Vegetation: Subtropical Dry Forest, with no evident anthropogenic disturbances.

Associated species: *Bursera simaruba* (Burseraceae), *Bucida buceras* (Combretaceae), *Coccoloba diversifolia* (Polygonaceae), *Comocladia dodonaea*

(Anacardiaceae), *Ottoschulzia rhodoxylon* (Urb.) Urb. (Icacinaceae), *Randia aculeata* (Rubiaceae), *Vanilla barbellata* (Orchidaceae).

Population 4: Caña Gorda, Bo. Carenero, Guánica

The population was on the south-facing slope of the low coastal limestone hills on the north side of route 333 (Fig. 9). The location was reached by walking at 40° starting at km 5.5 and continuing the hill about 150-200 m from the km marker. The slope where the species occurred was relatively gradual and had a large population of candelabra cacti (*Pilosocereus royeri*).

The population consisted of two individuals with their bases about 7-8 cm apart (Table 1). The extent of occurrence and the area of occupancy was approximately 2.25 m². The nearest population to it was the Murciélago population, which was 1.32 km (map distance) away.

The two individuals were relatively large (5 to 5.5 m in height) (Table 1). Both individuals were observed in flower during summer 2003 and one fruit was observed on #391 in October 2005. No seedlings or old fruits were found in the area.

Characteristics of the site include:

Substrate: Oligocene limestone

Coordinates: 17° 57' 17.7" N, 66° 53' 30.1" W

Elevation: 50 to 60 m

Aspect: South-facing slope.

Light conditions: Full sun.

Vegetation: Subtropical Dry Forest. Burning occurs frequently along Route 333 and extends up the slopes of the hills. In places extensive areas of native woody vegetation has been replaced by introduced grasses, primarily *Cenchrus ciliaris* L.

Associated species: *Hylocereus trigonus* (Haw.) Safford (Cactaceae), *Pilosocereus royenii* (Cactaceae), *Pithecellobium unguis-cati* (L.) Benth., (Mimosaceae), *Randia aculeata* (Rubiaceae).

Population 5: Trichilia Canyon (Cañón de las Trichilias), Bo. Carenero, Guánica

(Table 1; Figs. 9-11)

The population is in a north-south oriented canyon that is north of Cóbana Road (Fig. 9). The canyon has been named by the people at the Mayagüez herbarium for the large number of individuals of *Trichilia triacantha* found there. On some older herbarium labels it is referred to as part of the La Barina Trail. The junction between the trail to the canyon and Cóbana Road is 675 m east of Route 334 as the crow flies.

The population consisted of four plants varying from saplings less than 1 m tall with a stem less than 1 cm in basal diameter to plants 1.5 to 3.5 m tall that were sterile, but are assumed to be large enough to produce flowers and fruits. Two of the individuals occurred close together, having the same coordinates; the other two were isolated, with one 135 m and the other 250 m from its nearest neighbor. The extent of occurrence formed a triangle comprising 11,700 m². The area of occupancy was estimated to be 8 m². The nearest neighbor for the population was the El Cedro population, which was 2.18 km (map distance) away.

Characteristics of the sites include:

Substrate: Oligocene limestone

Coordinates: 17° 59' 19.6" N, 66° 51' 59.8" W

Elevation: 85 to 90 m

Aspect: On the west-facing slope of a north-south orientated canyon.

Light conditions: Shade in understory with dense canopy cover.

Vegetation: Subtropical Dry Forest, with evidence of disturbance due to flooding along the bottom of the canyon and hurricanes.

Associated species: *Celtis trinervia* Lam. (Ulmaceae), *Ottoschulzia rhodoxylon* (Icacinaceae), *Trichilia triacantha* (Meliaceae).

Population 6: Cedro Trail in Lomas de Seboruco, Bo. Carenero, Guánica (Table 1; Figs. 12-13)

The population was along a more or less southwest-northeast oriented trail off road 334. It consisted of seven plants varying from 1 to 2.5 m tall with one to three stems. Only one individual was found reproductive, having a single fruit. In addition to the seven adult individuals three seedlings were located around the individual. The extent of occurrence population was 3,004 m², forming two narrow triangles joined by a common group of individuals in about the middle; the area of occupancy was calculated to be 14 m², with the individuals scattered along a distance of approximately 435 m in length and varying from 22.5 to 154.5 m between nearest neighbors, with a mean distance of 67 m.

Characteristics of the site include:

Substrate: Oligocene limestone; soil very rocky.

Coordinates: 17° 59' 14.3" N, 66° 52' 51.8" W

Elevation: 100 to 125 m

Aspect: Very gentle northeast facing-slope.

Light conditions: Open shade of understory with thin canopy cover.

Vegetation: Subtropical Dry Forest. No evidence of recent disturbance.

Associated species: *Agave missionum* Trel. (Agaveaceae), *Tabebuia heterophylla* (DC.) Britton (Bignoniaceae), *Bucida buceras* (Combretaceae), *Bursera simaruba* (Burseraceae), *Coccoloba diversifolia* (Polygonaceae), *Croton betulinus* Vahl (Euphorbiaceae), *Eugenia ligustrina* (Sw.) Willd. (Myrtaceae), *Eugenia rhombea* (Berg.) Krug & Urb. (Myrtaceae), *Guettarda scabra* (L.) Vent. (Rubiaceae), *Gymnanthes lucida* Sw. (Euphorbiaceae), *Leptocereus quadricostatus* (Bello) Britton & Rose (Cactaceae), *Pictetia aculeata* (Vahl) Urb. (Fabaceae), *Pilosocereus royenii* (Cactaceae), *Pisonia albida* (Heimerl) Britton ex Standl. (Nyctaginiaceae), *Polygala cowellii* (Britton) S.F. Blake (Polygalaceae), *Randia aculeata* (Rubiaceae), *Thouinia striata* (Radlk.) var. *portoricensis* (Radlk.) Votava & Alain (Sapindaceae), *Tillandsia polystachya* (L.) L. (Bromeliaceae), *Trichilia triacantha* (Rutaceae), *Vanilla claviculata* (W. Wr.) Sw. (Orchidaceae), *Zanthoxylum flavum* Vahl (Rutaceae).

Flowering phenology

Flowering occurred between May and July in the two years (2003, 2004) of observations in the Barina population. In 2003 only individuals # 204, 244, 260 flowered; in 2004, six individuals flowered: # 204, 244, 260 (all of which flowered the previous year) and # 247, 252, and 253. Relatively few flowers were present at the beginning and towards the end of the flowering season.

Anthesis began at 7 a.m. with the lobes becoming fully spread by 3 p.m. The flowers remained open for 2 to 2 ½ days before abscission. The flowers produced a sweet fragrance that was strongest at 7 a.m. on the second day and weakest between 5 and 9 p.m. becoming noticeably stronger at 12 midnight. Nectar was present at anthesis and apparently throughout the life of the flower.

Flower morphology

Randia portoricensis has unisexual flowers and the plants are dioecious. The sepals are green, reduced to five small teeth. The corolla is salverform, 6-8 mm long, white at anthesis, becoming off-white with a pale tan tinge when past; the tube is cylindric, 5-7 mm long. The 5 corolla lobes are 1 mm long, ovate with acute apices and imbricate in bud. The staminate flowers have 5 white, versatile anthers, 2-3 (4) mm long with extrorse, distal dehiscence by a longitudinal slit. The pollen is white and shed in permanent tetrads (Figs. 14). The ovary is not reduced, but the ovules fail to differentiate (Fig. 15). In the female flowers the anthers produced no pollen and are black in color and more slender compared to those of the male flowers. The gynoecium is 7 mm long and green with the ovules clearly differentiated (Fig. 16).

When viewed through the fluorescence microscope the anthers and pollen from the male flowers emit yellow-green (Fig. 17), while the anthers from the female flowers emit orange (Fig. 18).

Fruits and germination

There were observations on fruit production for two individuals in the Barinas population: 244 and 247. Plant number 244 produced approximately 40 fruits each year in the fruiting seasons of 2002-2003 and 2003-2004; in 2004-2005 it produced only 10 fruits. Plant number 247 produced no fruits in 2002-2003 and 2003-2004, but produced approximately 40 fruits in 2004-2005.

Field observations showed the following stages in fruit ripening: immature fruits at maximum fruit size were green with a hard pericarp. The seeds were off-white with a pale tan tinge and were hard throughout. The seeds were tightly packed together in four groups of 6 to 8 seeds per group; soaking them in water for two days failed to separate the seeds. Seeds tested at this stage of development failed to germinate (Table 2). A small amount of colorless liquid was present in the space between the inner wall of the perianth and the seeds. When the fruit was cut and the liquid exposed to the air, it became dark bluish-black and readily stained any absorbent surface. Seeds exposed to the air changed from whitish to dark blue-black over a period of a few hours. The color did not fade over the observed time period of a month.

In older, but still immature fruits, the pericarp was grayish in color, but still remained hard. The seeds were tan to grey in color. They were moderately hard in texture, but could be bent without breaking. Two days of hydration failed to separate the

seeds. They became bluish-black when exposed to air. Seeds taken from fruits at this stage germinated, but required a minimum of 45 days for first germination to occur (Table 2) and germination ratios were low.

In mature fruits the pericarp was bluish-black to black in color and soft in texture. Mean length was 1.7 cm (s.d. = 0.1 cm, range 1.6 – 2.0 cm) and mean width was 1.3 cm (s.d. = 0.1, range 1.1 – 1.5 cm) (n = 13). This stage was reached approximately five months following pollination and would persist for approximately two months on the plant. The seeds were black in color throughout, and were soft except in the area of the embryo. The seeds were easily separated from one another following two days of soaking in water. The colorless liquid was no longer apparent, but a dust-like material occurred in the area between the pericarp and the seeds. Seeds collected from this stage of development germinated required a minimum of 17 days (Table 2) and 55% germinated.

Fruits older than five months that had fallen to the ground were dark in color, the outer layers of the pericarp had begun to disintegrate, and a circular opening appeared at the distal end of the fruit. Seeds at this stage were black and very soft, somewhat creamy in texture, and failed to germinate (data lost).

Seed number was fairly consistent per fruit, with a mean number of 24.2 seeds per fruit (s.d. = 1.64; range 24-26, n = 49, from two plants). Seed size varied from 4.0 – 5.5 mm in length, 2.5 – 4.5 mm in width and 1 – 2 mm in thickness.

Seedling growth and survivorship

In May 2004, 35 seedlings were found in the Barinas population growing in open shade under plant # 244. Mortality was not observed by 27 June 2005, when all 35 of the original juveniles, plus two more juveniles were counted. The seedlings were between 1.5 and 3.0 cm in height and occurred in tight groups of three to four individuals. The majority of seedlings were on the south side of the plant with the seedlings furthest from the plant extending to the east and west sides. Eleven of the seedlings were within a radius of 0.5 m from the base of plant # 244; 16 were 0.5 -1 m from # 244 and ten were within 1-1.5 m. No seedlings were found beyond a distance of 1.5 m.

Of the 11 seeds that germinated in test 2, 10 (91%) of the seedlings were still alive one year later and all of the 72 seedlings from seeds that germinated in test 3 were alive after one year (Table 3) in the greenhouse. However, in the summer of 2005, personnel problems in the greenhouse resulted in the death of a number of the plants due to lack of water. Only 50 individuals, all from the same parent plant, survived, and were subsequently transferred to the nursery at the Guánica Forest Reserve for planting out in Guánica Forest.

The seedlings from germination test 2, on the average, grew more than twice as tall as those from germination test 3, and had nearly four times the number of leaves and 12 times the number of branches. Germination, seedling and survivorship were observed to be better in light shade than in full sun. Growth results of the young plants were mixed: growth in height was similar in sun and shade, but the number of leaves was greater in the shade and plants in the full sun had yellow leaves vs. green ones on shade plants, which also branched earlier and had more branches.

greater in the shade and plants in the full sun had yellow leaves vs. green ones on shade plants, which also branched earlier and had more branches.

Seedling growth in the wild was slower compared to seedlings in the greenhouse, with the plants being shorter, having fewer branches and fewer leaves.

Informal observations showed that test 3 seedlings that germinated in partial shade produced more leaves and branches than those under full sunlight. When seedlings that had previously been in the shade were moved to full sun, their growth rate slowed and they produced fewer, smaller leaves.

DISCUSSION

The study resulted in the location of six populations with a total of 38 mature individuals. While this increased the number of known populations of the species from one to six, it has also shown a decrease in the number of individuals due to losses in the Barina population. In 1992, 41 mature individuals and 10 seedlings were marked in that population (Breckon and Kolterman 1994b). In 2002, only two of the marked adults could be located and none of the seedlings. Twenty-three mature, previously untagged individuals and 37 seedlings were found in an area adjacent to the area surveyed in 1992. Five of the adults disappeared between 2002 and 2005. If we assume that the 20 previously untagged individuals found in 2002 were present in 1994, then the original population size would have been 64 mature plants, of which 44 (69 %) were lost between 1992 and 2004. There are no data to determine if similar losses have occurred in the other five populations.

The island experienced a severe drought in 1994 (Anonymous, 1994) and the Barina area was hit by hurricanes Hortense in 1996 and Georges in 1998 (Ayala-Silva and Twumasi, 2004; Ramjohn, 2004). These climatic incidents could have impacted the population. Water stress has been cited as a primary factor in the regulation of the structure and dynamics of the tropical dry forest (Murphy and Lugo, 1986), but without data it is not possible to determine if climate negatively impacted the population and, if so, whether the small population sizes and the frequency of human disturbance acted to preclude recovery.

In April 2005, I encountered a man cutting trees, primarily *Guaiacum sanctum* L., for fence posts in the area of the Barina population. The stems of *Randia portoricensis* are too slender and spiny to be used for fence posts, but it is possible that plants of *Randia* were cut in order to clear the area to facilitate the cutting and removal of the trunks of the species cut for fence posts. That only tagged plants, all in the same area, disappeared suggests purposeful removal. This may account for the loss between 1992 and 2002. With the possible exception of the Caña Gorda population, the populations of *Randia portoricensis* were associated with populations of other rare species: *Trichilia triacantha*, *Ottoschulzia rhodoxylon*, *Polygala cowellii* and *Eugenia woodburyana*. *Ottoschulzia rhodoxylon* and *Eugenia woodburyana* occur in a canyon about 900 m east of the Caña Gorda population. The clustering of these rare species suggests that the areas were refuges from human disturbance.

Both of the locations on private land showed evidence of disturbance due to human activity. The owner of the land on which the Barina population occurred is planning to divide the property into lots for homes. The Peñón de Ponce population is also threatened by development. The land adjacent to it has been subdivided into lots and homes are being built on one side of the population, and a large gas station has been opened near the mouth of the canyon where the population occurs. The remaining four populations were found in the Guánica Forest Reserve. While they are protected, the Caña Gorda population was in an area where fires have often been set, resulting in a significant amount of the dry forest along road 333 being replaced by grassland dominated by introduced grasses.

It is difficult to evaluate the impact of the observed sex ratios, population size, and dispersion on the reproductive success of the species. The observed sex ratios should not be considered as the actual ratios in the population: female plants were determined primarily by the presence of fruits, which are long persistent on the plants. Male plants were determined by flowers, which are of relatively short duration. Finally, the occurrence of dioecy was not recognized until later in the study so that some plants are noted as flowering, but the sex was not determined. Without more information on the sex ratios and possible male parents, it is difficult to speculate on the genetic diversity of the seedlings from the different female plants. Fruits planted in the greenhouse produced clusters of seedlings and seedling clusters were observed in nature. The two plants in the Caña Gorda population could be from the same fruit, and if so, probably had the same pollen parent. Offspring from them would be equivalent to selfed offspring from a self-compatible bisexual individual.

The adult individuals varied from relatively clustered (as close as 7 - 8 cm) to highly isolated (1.32 km from the nearest neighbor). While population size can affect the ability to attract pollinators and seed dispersers, the impact depends on the species involved, the amount of reward offered and whether the surrounding species act as competitors or facilitators in attraction (Young and Brown, 1999; Murren, 2002; Lienert and Fisher, 2003). Flower morphology and timing sequences in *Randia portoricensis* strongly suggest nocturnal pollination, most likely by moths (Faegri and van de Pijl, 1979). Moths are a diverse group, but for pollination purposes they can be broken down into the noctuids, which tend to be small and flutter or alight rather than hover, and the

strong-flying sphingids that hover while visiting the flowers. At least some species of sphingids are capable of traplining and traveling over distances of several kilometers, which greatly expands the boundaries of the population and allows for more diffuse populations (Gill, 1988; Kato, 1996). The occurrence of a fruit on the apparently solitary individual of the Murciélago Canyon population, which was 1.32 km from the Caña Gorda population, is suggestive of traplining as no other individual of *R. portoricensis* was found in the area. The circumscription of “populations” as used in this work may require redefining. It is possible that the four Guánica populations may include one or several subpopulations linked by a pollinator. At one extreme then, the species consists of six discrete populations, and at the other extreme it has three populations, with the Guánica population composed of four subpopulations. Further, it would suggest that our inclusion of the isolated individuals within some of the populations (*i.e.*, Barina, Trichilia Canyon and El Cedro Trail) is justified.

The apparent absence of fruit dispersal suggests that either the disperser is extinct or that the populations are too small to attract dispersers. The fruits are enigmatic, being quite different from anything else in the flora, which makes it difficult to speculate as to a probable dispersal agent. The absence of dispersal away from the parent plant greatly reduces the plant’s ability to increase its local population size and all but precludes its ability to establish new populations.

The effect of population size on recruitment is hard to evaluate. Since the plants are dioecious a minimum of two individuals, male and female, should be required for fertilization. Vamosi and Otto (2002) hypothesized that dioecy could make plants more

vulnerable to extinction, using the low occurrence of dioecy among angiosperms (6 %) as possible evidence of either a poor ability to speciate or a high probability of extinction for dioecious species, but there is no other supporting evidence for their contention.

However, there is strong evidence that habitat fragmentation can have devastating consequences in dioecious and rare plants by isolating individuals, dispersers and pollinators (Young and Brown, 1999; Allen, 2000; Jutila and Grace, 2002; Murren, 2002; Lienert and Fisher, 2003; Liu and Koptur, 2003).

No evidence of either flowering or fruiting was found in the *Trichilia* Canyon population, which consisted of four individuals growing in deep shade and ranging from 2 to 125 m apart. Fruits were found in the other five populations, whose numbers ranged from one to 35 individuals, but seedlings were present only in the Barinas, Caña Gorda, and Cedro Trail populations. Given the short duration of the study, it is not possible to tell the frequency of seedling establishment or the life span of seedlings.

The restriction of the species to limestone within the Subtropical Life Zone could either be due to habitat specificity or to its elimination from other areas (see Cedeño-Maldonado and Breckon, 1996 for a discussion of the problem of determining edaphic endemism in Puerto Rico). The morphology of the plant indicates adaptation to dry conditions (small leaves, spines, slender stems), but not necessarily to limestone substrate. There was no evidence of habitat specificity within the dry forest. Individuals occurred on north-, northeast-, west- and south-facing slopes and in canyon bottoms. Plants were found at elevations between 50 and 130 m. The plants were growing in full sun and in open to fairly dense shade, with the vast majority of the individuals in partial

shade. The individuals in the densest shade were in Trichilia Canyon and they showed no sign of reproduction.

A species only needs to meet one of the five IUCN criteria established by The World Conservation Union to be considered as critically endangered (http://www.redlist.org/info/categories_criteria2001). *Randia portoricensis* meets three of them [B 1ab (v) + 2ab (v); C 1 + 2a (i); D]. It meets Criterion B1ab (v) as it has a geographic range with an extent of occurrence of less than 100 km² (estimated 22-25 km²) as calculated on the map. I am assuming that severe fragmentation has occurred as both substrate and climate are continuous in the area and would have resulted in numerous suitable habitats before human intervention. I observed a marked decline in the number of mature individuals in the one population (Barina) for which data were available. Using the same data from Criterion B1ab (v) the species also meets Criterion B2ab (v), by having a geographic range with an area of occupancy less than 10 km² (estimation 0.164 km²) with severe fragmentation, and a continuous decline of mature individuals. The species also meets Criterion C1, in having a population estimated to be less than 250 individuals (38 known adults) with a continuing estimated decline of 25% within three years or one generation, up to a maximum of 100 years. And using the same data that applied in Criterion C1, it also meets Criterion C2a (i) in having a continuing observed decline of mature individuals with a population structure with no more than 50 individuals in a single subpopulation or population. And finally it meets Criterion D in having less than 50 mature individuals.

The criteria used by the United States Fish and Wildlife Service are more subjective, with the primary emphasis being on whether the species is threatened with extinction in the near future (<http://www.fws.gov/>). Clearly, *Randia portoricensis* has little chance for survival given its presence status and its decline since 1992. Of the six populations, the two on private land are threatened with extirpation by human perturbation and development and one of the populations on the public land is threatened by fire. Of the 82 known adults in all populations, 44 (54 %) have died between 1992 and 2005. If we consider only the Barina population, then the loss is 69 %. The four populations on public land have a total of 14 individuals, with recruitment at the seedling level occurring in only two of them. Saving the species will require intensive management primarily by supplementing recruitment in existing populations, and the establishment of new populations.

Enhancement of existing populations and the establishment of new populations will be required to ensure the survival of *Randia portoricensis*. Both methods will require collection of wild seeds and production of nursery stock. The results indicate that ripe fruits (soft pericarp, blue to blue-black in color) taken from the plant give the best germination rates (see Table 4 for propagation protocols). Germination, seedling survival and growth were best under filtered or light shade. The greenhouse observations were borne out somewhat in that all seedlings in the wild were observed under the parent plant, but this could also be a result of lack of dispersal combined with shade tolerance. The occurrence of saplings and small adults in the semi-shade of the dry forest suggest some degree of shade tolerance or even shade dependence. The plants in Trichilia Canyon,

which were in relatively deep shade, would indicate that the species is quite shade tolerant, with their small size and the absence of reproduction best explained as due to tolerance to the deep shade. Adequate water is critical at all stages, with high mortality occurring in the seedlings and young saplings whenever the pots were allowed to dry out.

The most common threats for recently reintroduced species in the temperate flora of the United States were competition with exotic grasses, small population sizes and limited distributions, seed and seedling predation, flooding and erosion, land use for recreational purposes, and habitat destruction (Bowles and McBride, 1995; Brumack and Fyler, 1995; Cully, 1995; Gordon, 1995; Guerrant, 1995; Johnson, 1995; McDonald, 1995). Of these, the greatest threat for *Randia portoricensis* would be habitat destruction and limited population size and distribution. The threat to the existing populations on private land would exclude them from any attempts at enhancement. At this time, enhancement should be restricted to the four small populations in the Guánica Forest Reserve.

The genetic diversity in the existing populations of *Randia portoricensis* is probably low. Small populations usually have lower genetic diversity (Young and Brown, 1999; Gitzendanner and Soltis, 2000; Cole, 2003; De-Walt and Hamrick, 2004) and are subjected to faster genetic drift than larger populations (Guerrant, 1995). While it is well documented that the larger the founding population the better the chance for success (Guerrant, 1995), the fact that all the available seedlings of *Randia portoricensis* come from the same parent plant seriously limits enhancement strategies. Using material from the same parent plant to create new populations or to enhance existing populations

could lead to a loss in genetic diversity or to genetic bottlenecks (Young and Brown, 1999). Some balance then has to be reached between increasing the number of individuals in the population while not swamping the existing genetic diversity by an overabundance of a genotype. There are no guidelines to follow and without any information on the population genetics of *Randia portoricensis*, there is no objective way to determine what the upper limits should be for the number of individuals of presumably the same genotype that can be introduced into an existing population without causing a decline in genetic diversity. The fact that the plants are dioecious increases the chance of more than one pollen parent for the progeny of a female individual, which would increase the chance of genetic variability among the seeds. I am taking a conservative approach in assuming a single pollen parent. What I am proposing is a limited increase in population numbers and at the same time to test possible protocols for the successful introduction of new individuals into the wild.

There are approximately 50 young plants available in the Guánica Forest Reserve nursery, which allows for 12 to 13 plants per population. Six individuals could be planted in each of the four populations after the first rains of the rainy season in 2006 (late August, early September). These individuals would be monitored periodically. There are several possible outcomes: if all of the individuals live, then some will have to be removed to prevent overloading the population with the introduced genotype. Natural death may reduce the number of introduced individuals, which may reduce the number that have to be artificially removed or preclude the removing any of the plants. If the number of successful introductions falls below the prescribed number for the population,

then more can be introduced in the 2007 rainy season. Extra plants could be used for educational plantings around the Reserve Headquarters or, if new stock from different parent plants becomes available, used in creating new populations.

In the Murciélagos population, I would recommend that no more than two new individuals be established, both of which should be near (1 - 3 m) the existing plant but away from each other (*i.e.*, in a straight line with the existing plant in the middle). For the Caña Gorda population two or three individuals could be established, again near the existing plants, but away from one another. The population at El Cedro Trail, with seven plants in linear arrangement, could absorb three to four individuals, each planted approximately midway between two existing individuals. Trichilia Canyon, with its dense shade, presents a more difficult scenario: placement there should be done in light shade where possible regardless of the position of the existing plants. The goal there would be for the establishment of two or three new individuals at scattered locations in the canyon. If the sex of the introduced plants and the sex of the existing individuals are known, then a balanced male/female sex ratio should be sought, and as much as possible with the plants placed so as to maximize mixed visitations by the pollinators. If successful, the plantings would add between nine and twelve new individuals to the forest, which still leaves the total number of individuals of the species below IUCN's 50 individuals' criterion, thus leaving *Randia portoricensis* Critically Endangered.

CONCLUSIONS

With the existing data about *Randia portoricensis* population, I concluded that according to the IUCN criteria, the species is a candidate for listing as Critically Endangered. Pollination was not determined during the study, but according to the flower morphology the species may be pollinated by sphingids, but there is not enough evidence to justify the argument. The distribution of seedlings in the wild may suggest a lack of dispersers, but again not enough evidence was found to support this hypothesis. According to the existing information, the species prefers limestone substrates, shady conditions, and association with other rare species, but this statement may need to be redefined. Through propagation studies I concluded that seed germination is strongly dependent by fruit maturity and stage of planting.

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Table 1: Coordinates, elevation, tag number, height, diameter, stem number and reproductive status of individuals of *Randia portoricensis*.

Locality	GPS Location	SA (m ²)	Elev. (m)	Tag #	Height (m)	TBD (cm)	Stems #	Sex
Population 1	18° 01' 0.66" N / 66° 49' 45.5" W	96	110-130	201	3	6.7	2	--
	18° 01' 3.36" N / 66° 49' 46.6" W	96	110-130	204	1	0.9	2	*
	18° 01' 3.84" N / 66° 49' 41.1" W	96	110-130	242	0.4	1	1	--
	18° 01' 3.84" N / 66° 49' 41.1" W	96	110-130	243	0.8	1.1	1	--
	18° 01' 3.84" N / 66° 49' 41.1" W	96	110-130	244	4.5	7.8	9	♀
	18° 01' 3.84" N / 66° 49' 41.1" W	96	110-130	245	1	1.6	2	--
	18° 01' 2.82" N / 66° 49' 43.8" W	96	110-130	246	0.5	0.9	1	*
	18° 01' 2.82" N / 66° 49' 43.8" W	96	110-130	247	2.5	3.6	1	♀
	18° 01' 2.82" N / 66° 49' 43.8" W	96	110-130	248	1	1	1	--
	18° 01' 3.60" N / 66° 49' 42.2" W	96	110-130	249	2	2	2	♀
	18° 01' 2.88" N / 66° 49' 43.9" W	96	110-130	250	1.5	0.7	1	--
	18° 01' 2.88" N / 66° 49' 43.9" W	96	110-130	251	1.5	2.2	1	♀
	18° 01' 2.88" N / 66° 49' 43.9" W	96	110-130	252	1.5	1.8	2	--
	18° 01' 3.00" N / 66° 49' 43.9" W	96	110-130	253	0.3	<1	1	*
	18° 01' 3.00" N / 66° 49' 43.5" W	96	110-130	254	1	<1	3	--
	18° 01' 3.66" N / 66° 49' 41.6" W	96	110-130	255	1	1.6	2	--
	18° 01' 4.62" N / 66° 49' 39.5" W	96	110-130	256	1.5	2.7	2	♀

Table 1: Cont.

Locality	GPS Location	SA (m ²)	Elev. (m)	Tag #	Height (m)	TBD (cm)	Stems #	Sex
Population 1	18° 01' 4.62" N / 66° 49' 39.5" W	96	110-130	257	1	1.3	1	--
(cont.)	18° 01' 4.62" N / 66° 49' 39.5" W	96	110-130	258	1	0.8	1	--
	18° 01' 4.62" N / 66° 49' 39.5" W	96	110-130	259	1	<1	1	†
	18° 01' 13.6"N / 66° 49' 37.9" W	96	110-130	260	5	8.2	8	♂
	18° 01' 13.6"N / 66° 49' 37.9" W	96	110-130	261	1	<1	1	†
	18° 01' 13.6"N / 66° 49' 37.9" W	96	110-130	262	1	<1	1	†
	18° 00' 57.4" N / 66° 49' 43.5" W	96	110-130	263	3.5	<1	2	†
	18° 00' 57.4" N / 66° 49' 43.5" W	96	110-130	264	1.5	<1	1	†
Population 2	17° 59' 20.7" N / 66° 42' 16.8" W	10	50	1‡	2	3	2	♀
	17° 59' 20.7" N / 66° 42' 16.8" W	10	50	2‡	3	1.5	1	--
	17° 59' 20.7" N / 66° 42' 16.8" W	10	50	3‡	2.5	2.5	1	--
	17° 59' 20.7" N / 66° 42' 16.8" W	10	50	4‡	1.5	2.2	1	--
Population 3	17° 57' 58.0" N / 66° 51' 46.9" W	2	70	389	5	2.3	1	♀
Population 4	17 57' 17.7" N / 66° 53' 30.1" W	2.25	50-60	390	5.5	5.5	2	♂
	18 57' 17.7" N / 66° 53' 30.1" W	2.25	50-60	391	5.5	4.1	1	♀

Table 1: Cont.

Locality	GPS Location	SA (m ²)	Elev. (m)	Tag #	Height (m)	TBD (cm)	Stems #	Sex
Population 5	17° 59' 11.1" N / 66° 52' 01.1" W	8	85-90	387	1.5	1.2	1	--
	17° 59' 19.6" N / 66° 51' 59.8" W	8	85-90	388	3.5	3.9	2	--
	17° 59' 19.6" N / 66° 51' 59.8" W	8	85-90	392	<1	<1	2	--
	17° 59' 23.1" N / 66° 51' 56.0" W	8	85-90	393	1.5	1.2	1	--
Population 6	17° 59' 08.0" N / 66° 52' 50.3" W	14	100-125	394	1	<1	1	--
	17° 59' 12.9" N / 66° 52' 51.8" W	14	100-125	395	2	2.6	2	--
	17° 59' 14.3" N / 66° 51' 51.8" W	14	100-125	396	2	2.9	3	--
	17° 59' 16.6" N / 66° 52' 52.8" W	14	100-125	397	1.5	1.1	1	--
	17° 59' 19.2" N / 66° 52' 53.1" W	14	100-125	398	1.5	1.4	1	--
	17° 59' 19.9" N / 66° 52' 53.1" W	14	100-125	399	1.5	1.6	2	--
	17° 59' 22.1" N / 66° 52' 53.3" W	14	100-125	400	2.5	3	1	♀

Abbreviation and symbols: SA = study area; Elev. = elevation; Tag # = identification number for individual according to the tag assigned; TBD = total basal diameter; #‡ = no assigned tag, individuals were labeled from 1 through 4 (population 2); -- = unknown sex; * = flowers observed, but sex not determined; ♀ = female individual; ♂ = male individual; † = individual disappeared, assumed to be dead. The height of the individuals was estimated. Individuals less than 1 m height were measure, while heights larger than 1 m height were estimated. TBD was calculated with the following equation: $TBD = \sqrt{(i_j^2 + \dots + i_j^2)}$, where i is the corresponding DBH (diameter at breast height for main stem or stems).

Table 2: Seed germination rate and percentage from fruits at different stages of maturity.

Fruit Stage	# Seeds	Medium	Time (days)	1 st Germ. (days)	Last germ. (days)	% Germ.
Immature green fruits	25	FPPD	70	0	0	0
Immature gray fruits	90	CSM	90	45	70	12
Mature bluish-black fruits	150	CSM	94	17	94	55

Abbreviations and symbols: FPPD = Filter Paper in Petri Dishes; CSM = Commercial Soil Mix; Germ. = Germination.

Table 3: Seed germination and seedling survivorship and growth in seeds from fruits at different stages of maturation.

GT	N	Germ.	Germ. %	Seedlings Alive	Survival %	\bar{x} # Lvs.	s.d.	\bar{x} # Branches	s.d.	\bar{x} Height	s.d.
1	25	0	0.0	*	*	*	*	*	*	*	*
2	90	11	12.2	10	90.9	39.8	6.4	7.5	1.1	11.4	4.1
3	150	72	48.0	72	100.0	10.1	5.6	0.6	1.6	5.0	3.0
Barinas	*	*	N/A	35	100.0	7.0	3.3	0.1	0.3	3.1	1.4

Abbreviations and symbols: GT = germination test, 1 = immature green fruits, 2 = immature grey fruits, 3 = mature bluish black fruits; N = sample size (number of seeds planted); Germ. = germination; Germ. % = germination percent; \bar{x} # Lvs. = average number of leaves; S.D. = standard deviation; \bar{x} # Branches = average number of branches; \bar{x} Height = average height; and * = unknown data to determine number of seeds available in the soil, how many seeds germinated out of the original source.

Table 4: Protocols for the propagation of *Randia portoricensis* in the nursery.

Protocol	Activity
1	Ripe fruits (pericarp blue to blue-black and soft) should be picked from the parent plants.
2	Fruits should be marked as to parent with fruits from different parents kept separate.
3	The seeds should be planted within a week of collection. They should be maintained under dry conditions at room temperature in paper bags until planted.
4	Soak the fruits for two days in water before removing the seeds. This will make separation of the seeds easier and will hydrate the seeds before planting.
5	Once hydrated the seeds can be separated by gently squeezing them with your fingertips. (Latex gloves should be worn to prevent the fruit from staining your fingers.)
6	Seeds should be planted in a good commercial planting soil. Vermiculite should be avoided as it retains excessive amounts of water. The medium should be kept moist with the pots in filtered shade.
7	Seeds should be labeled as to date of planting, population and parent plant. This information must be kept with each plant to prevent plants with the same parentage from being planted together when they are planted out.

Cont.

Protocol	Activity
8	The seedlings should be transferred to 6 inch pots when they start to branch. They are sensitive to root damage, so care must be taken to disturb the roots as little as possible and to try to maintain the root ball.
9	The transplanted seedlings should be watered immediately and then monitored at least twice a day for the first two days to prevent drying out.
10	Seedlings and saplings should be maintained under filtered or light shade in the greenhouse and nursery. Adequate water is critical. They are very sensitive to drying out, which is the major cause of mortality in the greenhouse material.



Figure 1: Key characters for the identification of *Randia portoricensis* from other species of the genus *Randia*. Letter A represents the flower of the species (see text for description). B represents the spines arrangement which form a trichotomy of three terminal spines. C represents the almost sessile leaves which characterize both, *Randia aculeata* and *Randia portoricensis*. The picture was taken from the individual # 247 of the Barina population.



Figure 2: Branch of *Randia portoricensis* with an immature green fruit. Letter A indicates the fruit. Picture taken from individual # 247 from the Barina population.



Figure 3: Mature bluish-black fruit used for germination test 3. The fruit corresponds to individual # 247 of the Barina population.

Figure 4: Location of populations of *Randia portoricensis* found between 2002 and 2005. The locations for the populations are marked in red. Population 1, Barina; population 2, Peñuelas; population 3, Cañón de los Murciélagos; population 4, Caña Gorda; population 5, Trichilla Canyon; population 6, Cedro Trail.

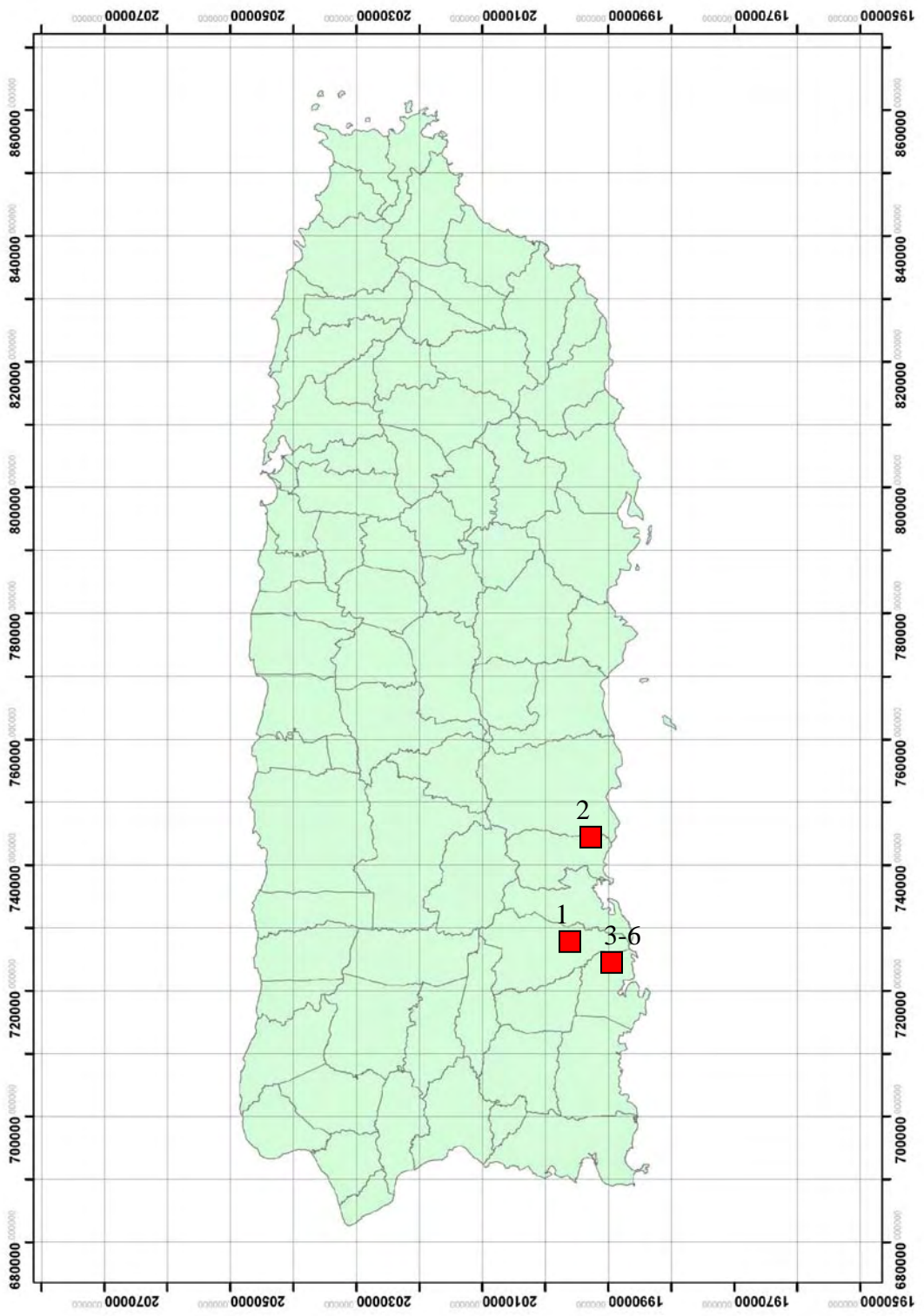


Figure 5: Distribution of individuals in the Barina population. The quadrangle approximately outlines the area of the population. The cross inside the quadrangle marks the GPS location for the population.

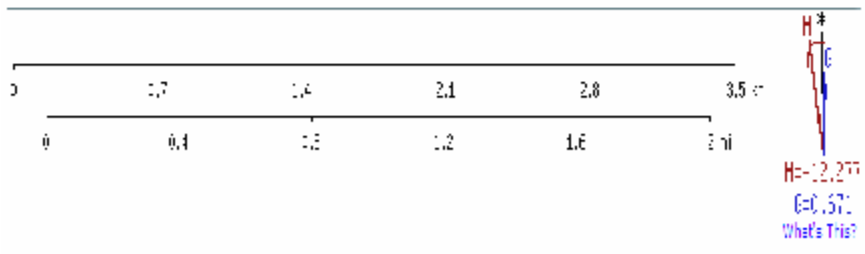


Figure 6: Distribution of individuals in the Barina population. Abbreviations and symbols: square = six clustered individuals. Black circles represent non-reproductive individuals. Asterisk represents reproductive individuals. One of the clustered individuals is a female; # 247. The number adjacent to the symbols represents the number of individuals that share the same coordinate (for specific coordinates of each individual see table 1). The two lines between black circles represent the distance between those individuals.

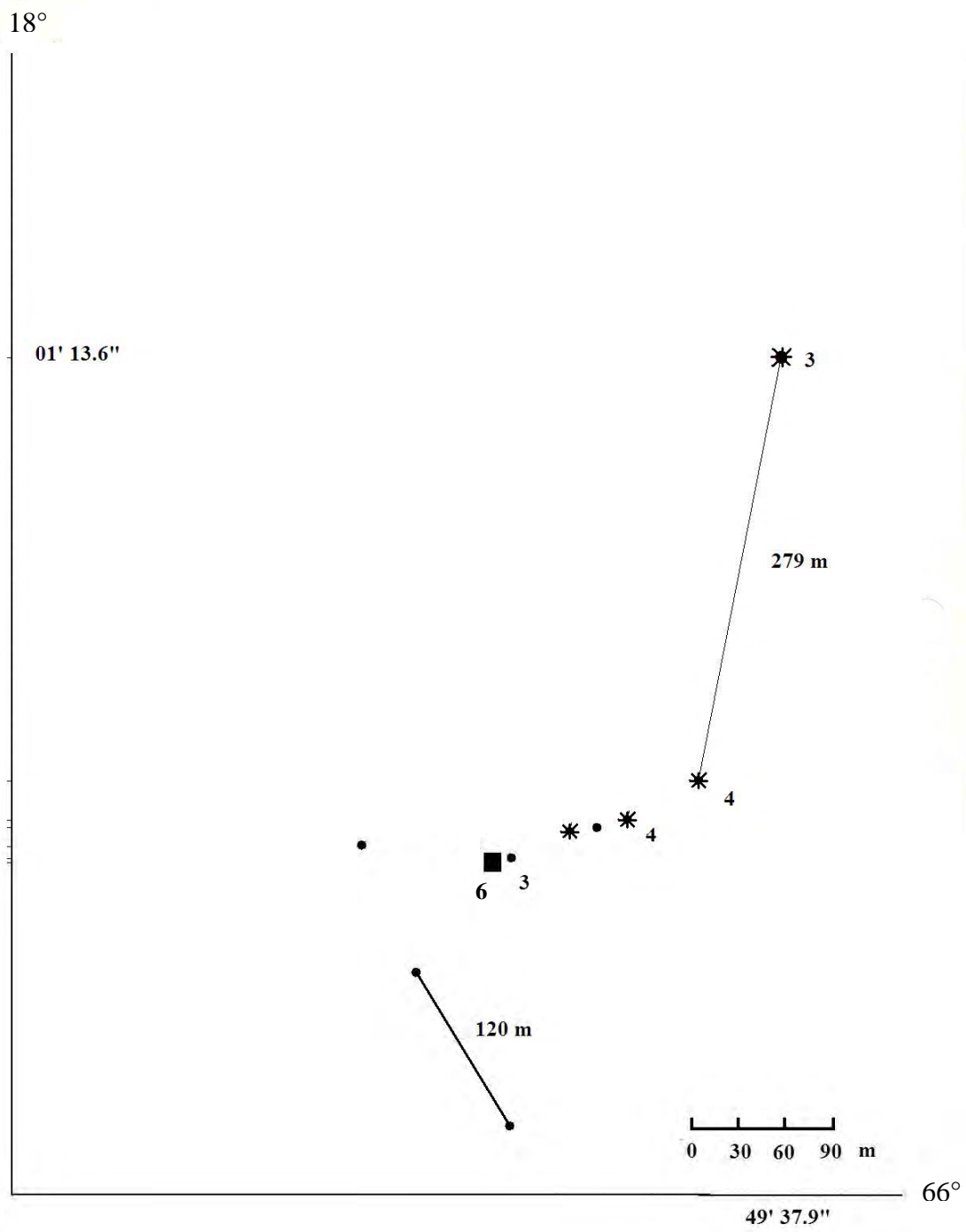




Figure 7: Location of the Peñuelas population at the Peñón de Ponce (indicated by the open square). There are four individuals in the bottom of a north-south canyon that periodically has running water.

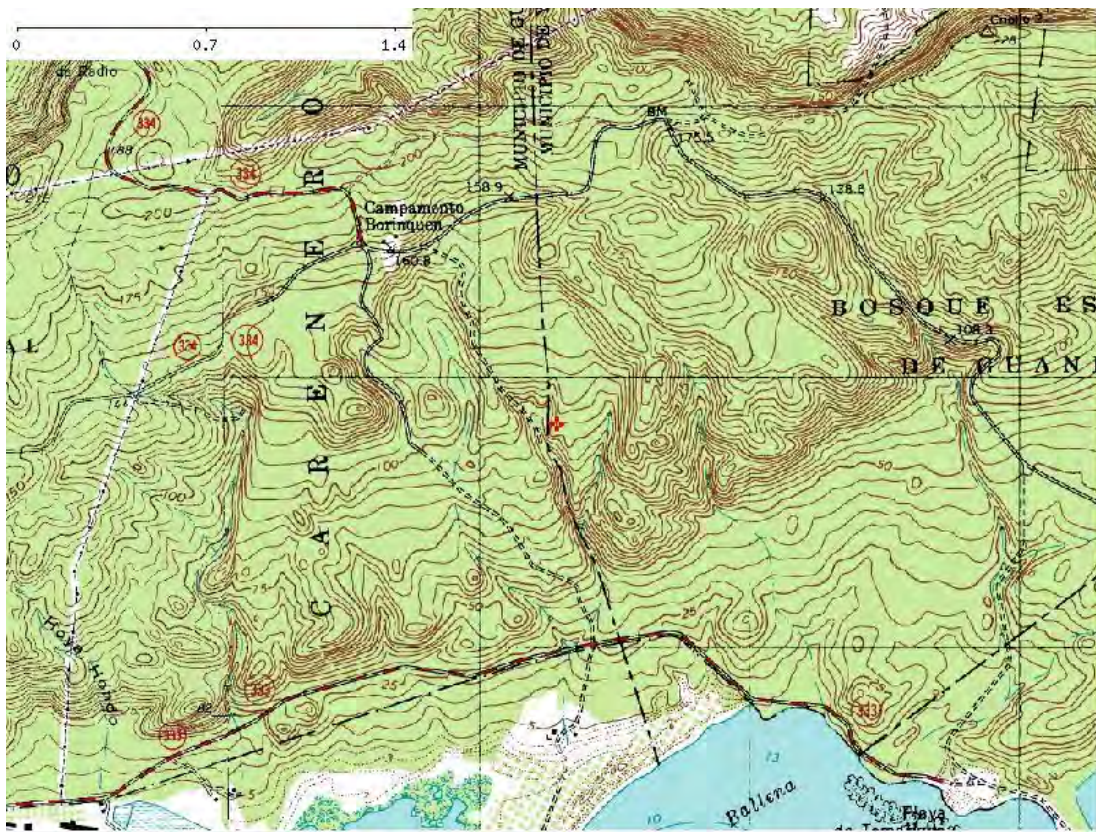


Figure 8: Location of Cañón de los Murciélagos population in Guánica Forest Reserve (indicated by the cross). One individual was found.

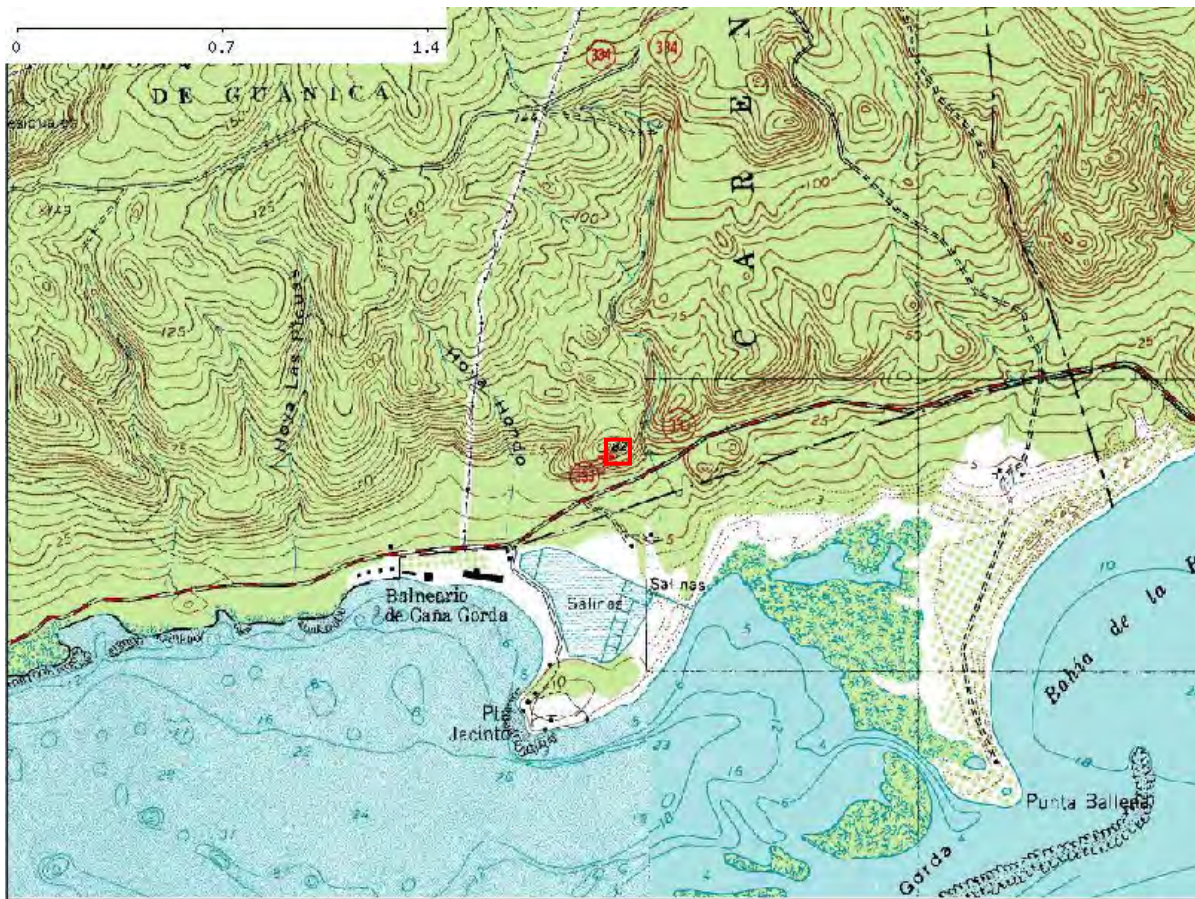


Figure 9: Location of the Caña Gorda population in the Guánica Forest Reserve (indicated by the open square). The two individuals are 25 cm from one another.

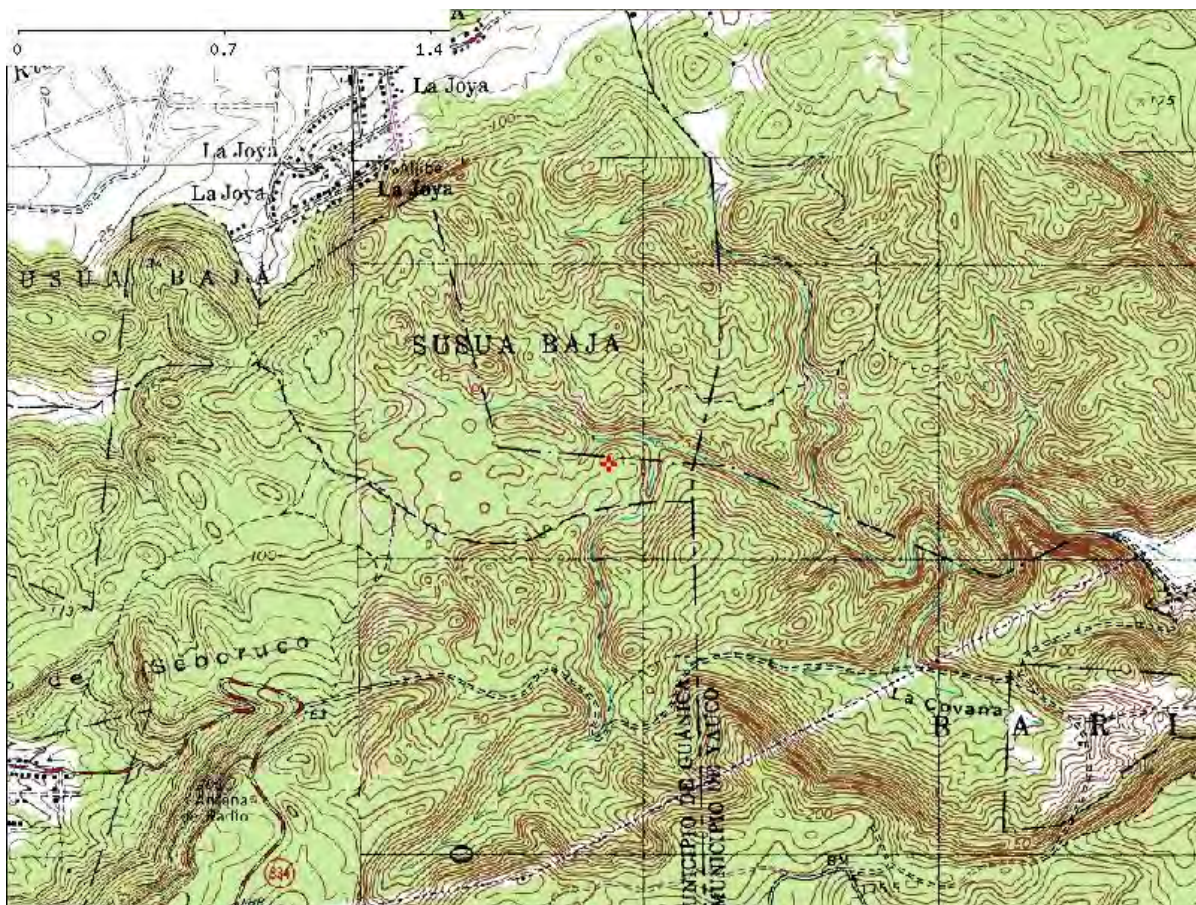


Figure 10: Location of the *Trichilia* Canyon population in the Guánica Forest Reserve (open rectangle). Four individuals were located, all sterile.

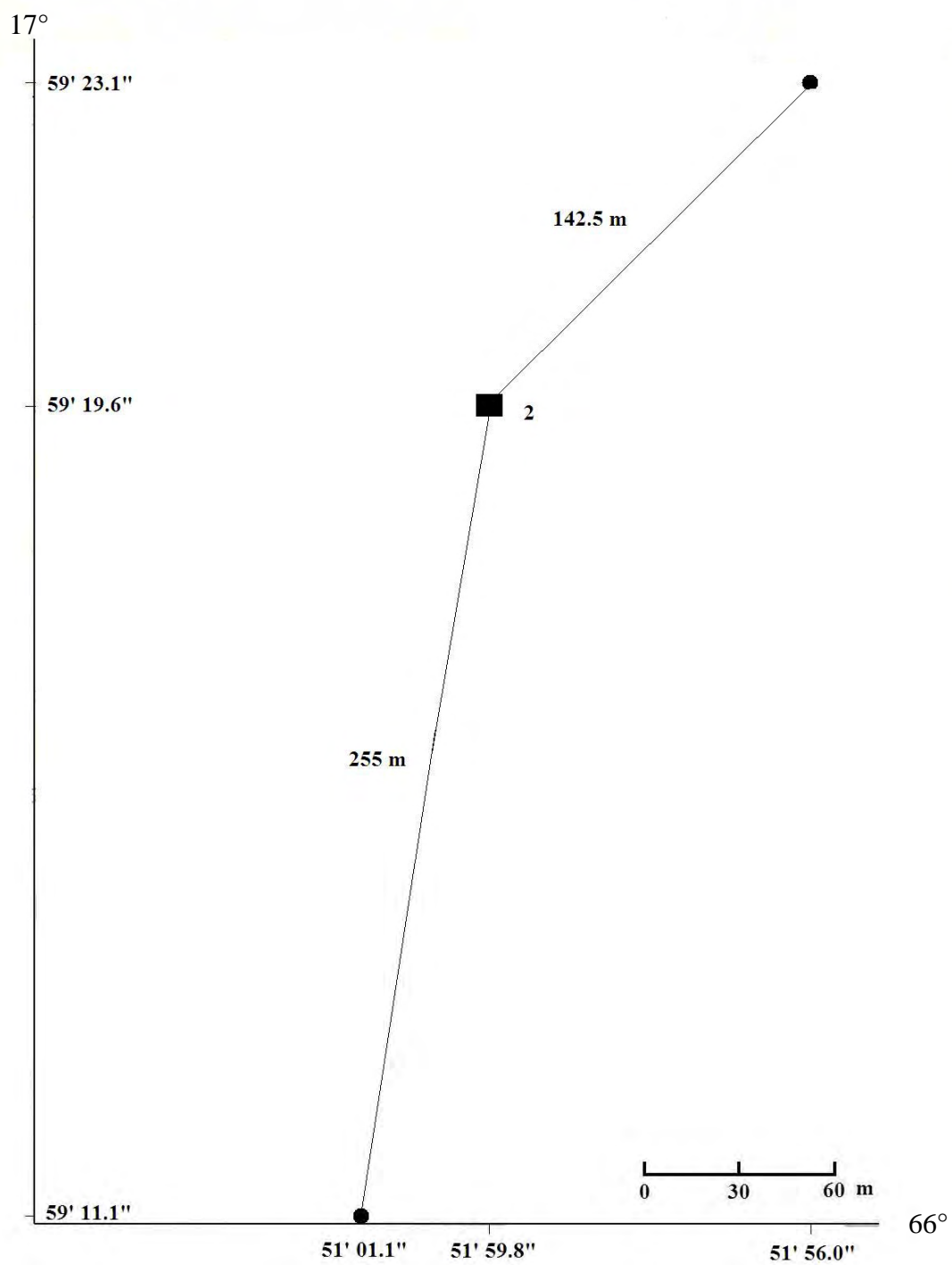


Figure 11: Distribution of individuals in the Trichilia Canyon population. Symbols: Black square indicates two individuals. Circles are solitary individuals.

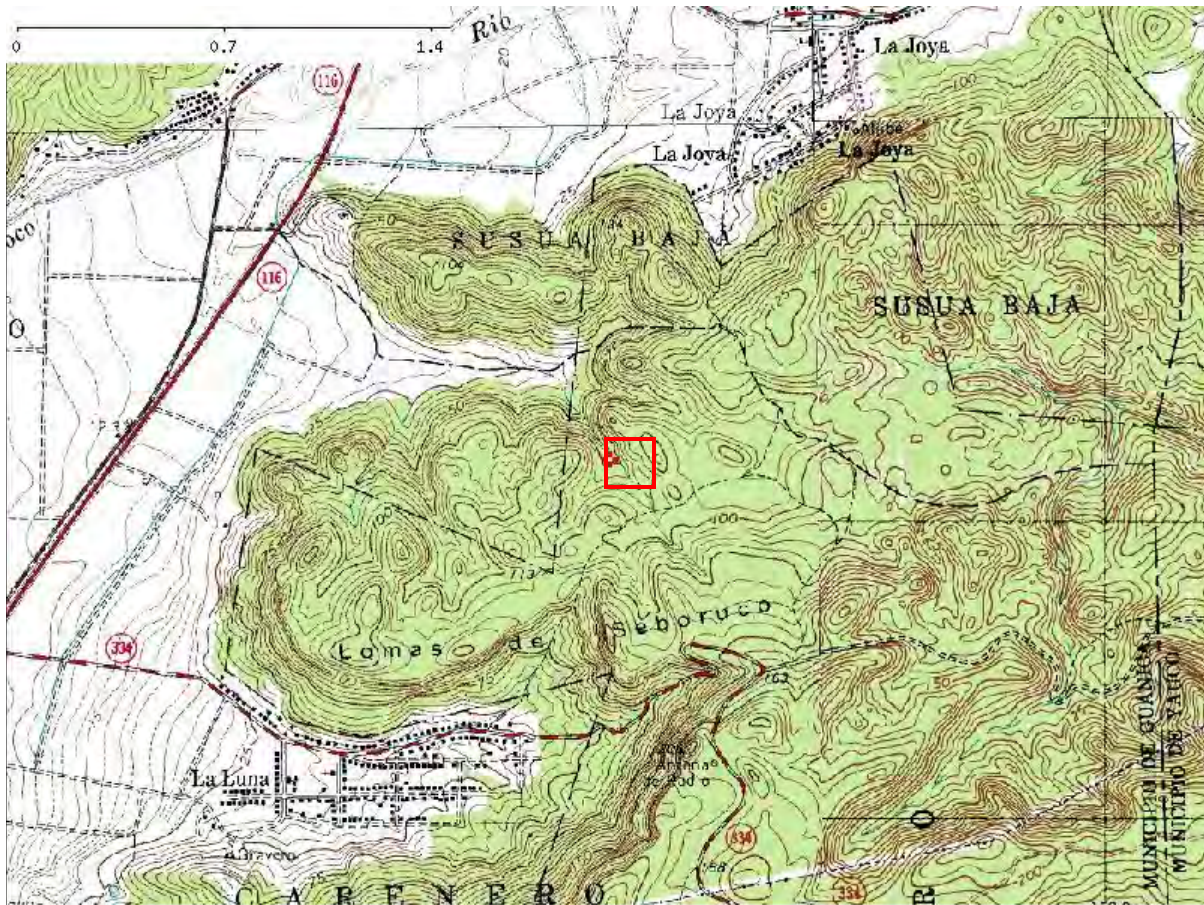


Figure 12: Location of the Cedro Trail population in Lomas de Seboruco, Guánica Forest Reserve (open rectangle).

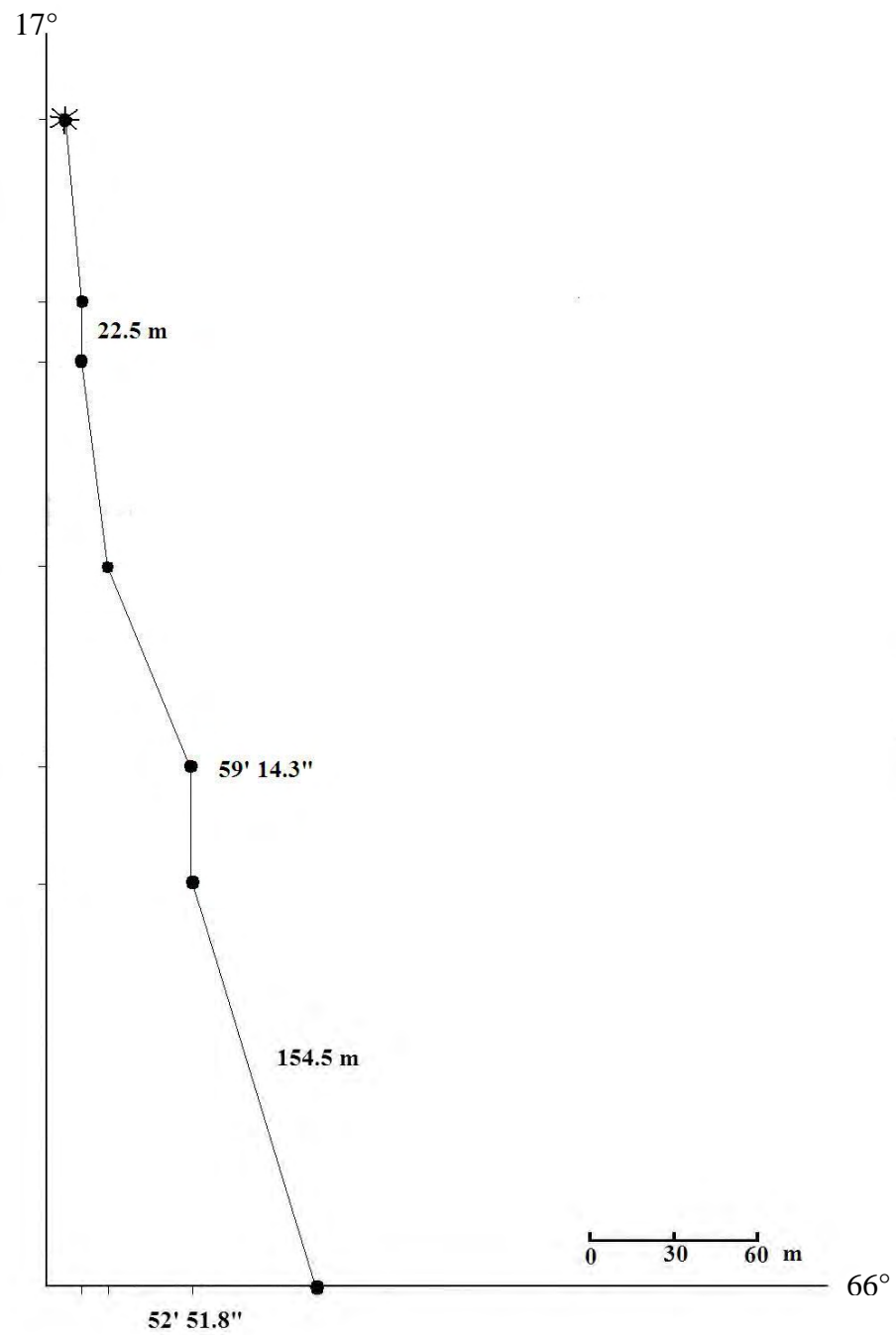


Figure 13: Distribution of individuals in the Cedro Trail population. Asterisk indicates reproductive individual; circles non-reproductive individuals. The distances between individuals varied from 22.5 to 154.5 m.



Figure 14: Viable pollen tetrads from individual 260 in Population 1. The viable pollen grains emit a yellow-green fluorescence, which was digitally modified to orange for resolution purposes. Permanent tetrads are characteristic of the genus *Randia*.



Figure 15: Dissection of a male flower of *Randia portoricensis* showing undifferentiated ovules. Flower from individual # 260.

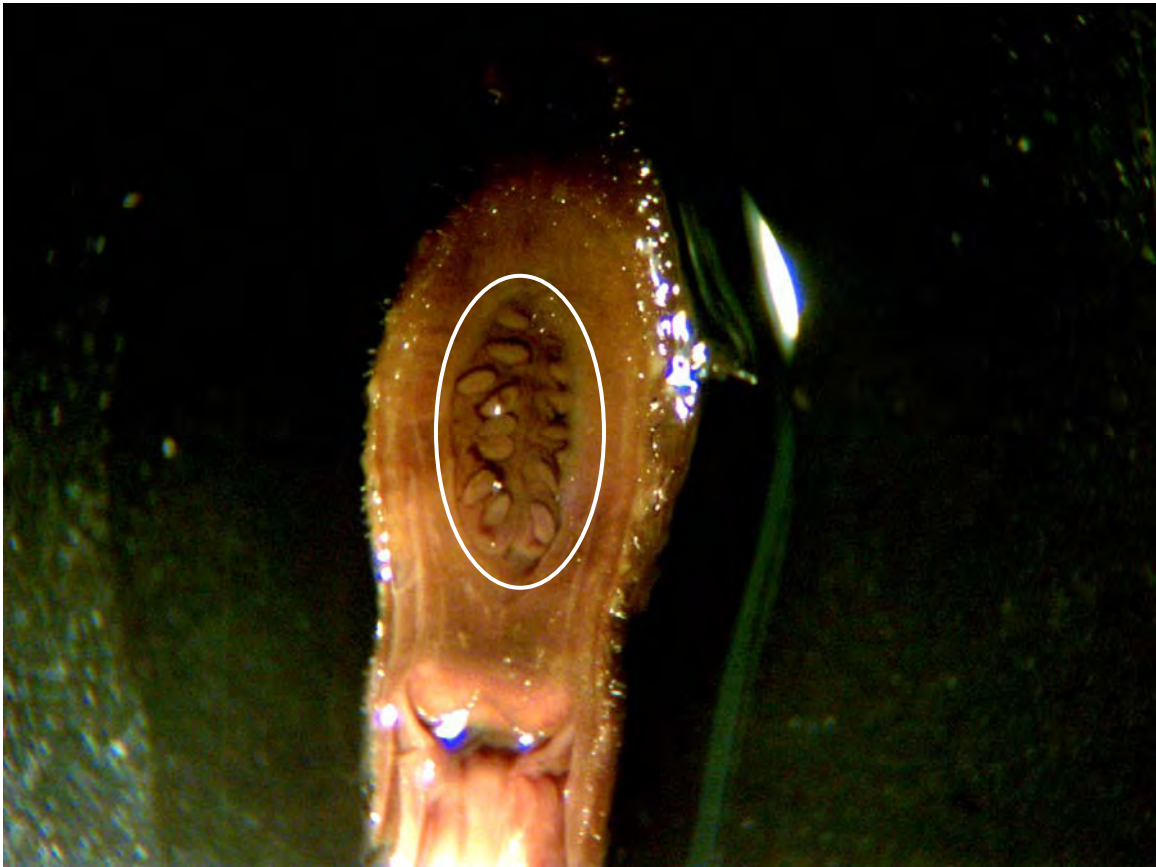


Figure 16: Differentiated ovules in the ovary of a female flower. The flower was collected from individual # 247.

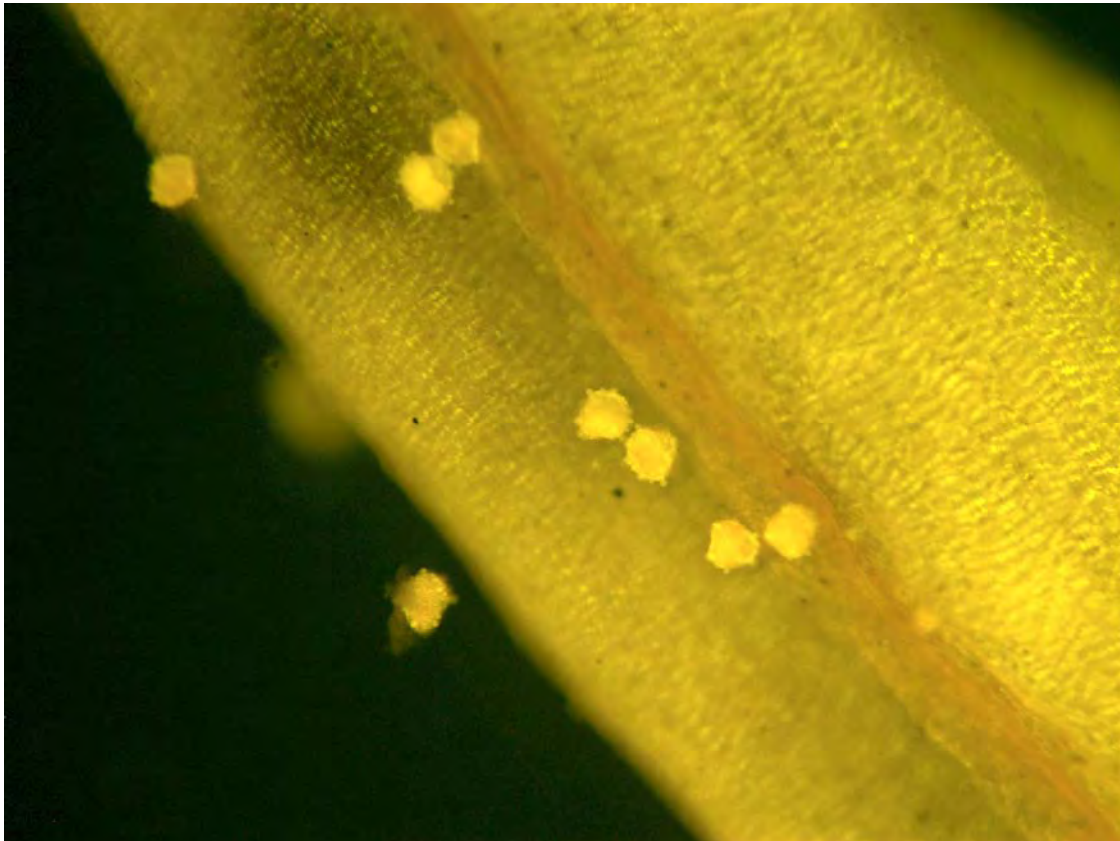


Figure 17: Yellow-green emission by anthers from male flowers when viewed under the fluorescence microscope with 10X objective. The flower was collected from individual # 260.



Figure 18: Orange fluorescence emitted by anther from a female flower bud. In the absence of pollen grains, the anthers do not emit yellow-green fluorescence. The flower was collected from individual # 247.