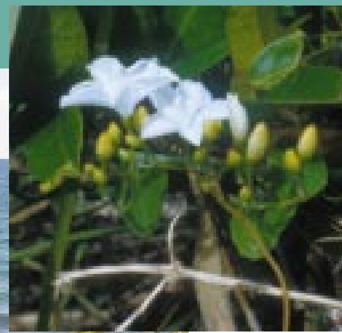

Shade limited root mass and carbohydrate reserves of the federally endangered Beach Clustervine *Jacquemontia reclinata* grown in containers

Photo by Joyce Maschinski | Inset photo by Hannah Thorton

ABSTRACT

Anecdotal evidence suggested that germination and seedling growth of the federally endangered beach clustervine (*Jacquemontia reclinata* [Convolvulaceae]) were best in the shade, but mature plants usually occur in coastal strand areas that are open or have low vegetation. We conducted an experiment using potted seedlings grown without shade, or under low, moderate, or heavy shade enclosures. Shade did not affect shoot growth, or leaf or stem dry mass. Plants subjected to all levels of shade, however, had 40% to 70% less root dry mass and about 50% lower root-to-shoot ratios than non-shaded plants. Moreover, the roots of non-shaded plants had 2 to 4 times more soluble sugars and starch than plants grown in shade. These findings suggest that when water and nutrition are not limiting, *J. reclinata* seedlings are best grown without shade.

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KEY WORDS
revegetation, coastal strand, propagation, light

NOMENCLATURE
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Figure 1. Federally endangered beach clustervine (*Jacquemontia reclinata* [Convolvulaceae]) blooming in its natural habitat.

Beach clustervine (*Jacquemontia reclinata* House) is a perennial vine of the morning glory family (Con-volvulaceae) endemic to the coastal strand of southeastern Florida (Figure 1). Disturbance and fragmentation of beach clustervine's habitat by commercial and residential developments (Figure 2) have further restricted its range and contributed, in part, to its status as a federally endangered species (Fisher and Jayachandran 2002). Other factors contributing to beach clustervine's endangered status include low germination rates in the wild, competition from exotic or invasive plants, and narrowing of the coastal strand due to hardwood hammock encroachment and beach raking (Figure 3). Even within protected areas, *J. reclinata* populations are small and scattered. According to Lane and others (2001) only about 700 wild plants, most of which are from 2 populations, exist.

Environmental factors that might limit *J. reclinata* within the coastal strand are unknown, but evidence indicates that light levels may be involved. For example, mature *J. reclinata* plants are usually associated with low vegetation or open areas that are not shaded. Historically, periodic natural fires maintained a low tree and shrub cover in the coastal strand, but as people have suppressed fires in this area, tree and shrub cover has increased (Austin and others 1977; Wright 2003). In contrast to the apparent preference of mature plants for open areas, anecdotal evidence suggests that germination and seedling growth of *J. reclinata* in the wild is best in the shade (USFWS 1999). Under glasshouse conditions, however, sunlit seeds and seeds kept in the dark germinated equally well (Fisher 2003). The effect of shade on seedling growth of *J. reclinata* has not been tested, although seedlings of a related plant, small-flower morning glory (*Jacquemontia tamnifolia* [L.] Griseb.), grew best in full sun (Shaw and others 1987).

Because of beach clustervine's restricted range, small populations, and low rate of natural recruitment, conservation programs depend on propagated plants for use in research and reintroduction projects (USFWS 1999). Therefore, knowledge of the plant's environmental preferences in culture (Affolter 1997; Fisher and Jayachandran 2002), as well as in the wild, is needed. At Fairchild Tropical Garden, located in Coral Gables, Florida, plants may be propagated and maintained in the open, under shade cloth, or in glasshouses. The shade cloth and glasshouse facilities reduce light levels by 30% to 70% compared to full sun. Data regarding the most suitable light environment for cultivation of *J. reclinata* are lacking. Thus, we conducted an experiment to test the effect of low, moderate, or heavy shade on the development of *J. reclinata* seedlings to determine whether seedlings benefit from shade, and to identify facilities with the best light environment to propagate and maintain collections of this plant. A further goal was to determine suitable *J. reclinata* microhabitat conditions in order to increase survival of outplanted and wild populations.

MATERIALS AND METHODS

Plant Material

Seeds of *J. reclinata* were collected from open-pollinated plants of Fairchild Tropical Garden's *ex situ* conservation col-

Photo by Samuel J Wright

Figure 2. Coastal development threatens habitat for beach clustervine and other coastal species.

lection. Seeds were germinated in community pots, and 60 seedlings of uniform appearance and stem length (about 4 cm [1.6 in]) were selected for the experiment. Each seedling was transplanted into a separate 750-ml (11.2 cm [4.4 in] x 12.2 cm [4.8 in]) black polyethylene container (Landmark Plastics Corp, Akron, Ohio) filled with artificial medium (4:3:2:1.5; peat moss:silica sand:pine bark:perlite). Each pot was nested in another (pot-in-pot) to insulate the growing medium from solar heating (Martin and others 1999). At potting, all plants were topdressed with approximately 7 g (0.2 oz) of Nutricote (Florikam, Sarasota, Florida) controlled release fertilizer ($10N:10P_2O_5:17K_2O$; 6 mo release rate at 25 °C [77 °F]). Potted plants were placed outside on black polyethylene tables. Tap water was applied to plants of all treatments as needed, but frequent rain generally satisfied the plants' water needs.

Shade Treatments

Four levels of shade were tested: no shade (control), low shade (30% light exclusion by shade cloth), moderate shade (60% light exclusion), and heavy shade (about 90% light exclusion; 30% + 60% shade cloth). Each level of shade was established by placing potted plants within wood-framed enclosures (33 cm [13 in] tall x 1 m [39.4 in] wide x 1 m [39.4 in] long) covered with the appropriate shade cloth (Figure 4). Control plants were placed on bench spaces without chambers.

Growth and Mass Measurements

Shoot length and leaf number of each plant were measured weekly, and each plant was harvested after 47 d. At harvest, stems were separated from roots. Substrate was carefully removed from roots by hand. Shoots and clean roots were then oven dried at 65 °C (149 °F) for 2 d. Leaf, stem, and root mass of each plant was determined separately.

Carbohydrate Analyses

Levels of non-structural carbohydrates (fructose, glucose, sucrose, and starch) in the roots were determined for each treatment replicate. Dried roots were ground in a mill to pass through a 40-mesh screen. Soluble sugars were extracted from the ground tissue with hot deionized water and analyzed by HPLC with refractive index detection (Johansen and others 1996). After soluble sugars were extracted, the tissue was subjected to a solution of α-amylase and amyloglucosidase to digest starch and convert it to free glucose that was detected by HPLC (Smith 1969).

Experimental Design and Statistical Analysis

This was a single factor (level of shade) experiment in a randomized, complete block design with 3 replicates per treatment. Each treatment replicate consisted of 5 potted plants in a shade enclosure (shade treatments) or open table space of equivalent area (control). For most variables measured, data subjected to statistical analyses were the average of 5 plants per treatment replicate ($n = 3$). Because of low root mass, however, root systems within each replicate were combined for carbohydrate analyses; thus each treatment replicate consisted of a sample of homogenized root tissue of 5 plants. Data were subjected to analysis of variance, using the general linear model (GLM) procedure of SAS statistical software (SAS

Photo by Samuel J Wright

Figure 3. The management practice of beach raking has contributed to loss of habitat for native coastal plant species.

Photo by Samuel J Wright

Figure 4. Shade enclosures used for the beach clustervine shade study.

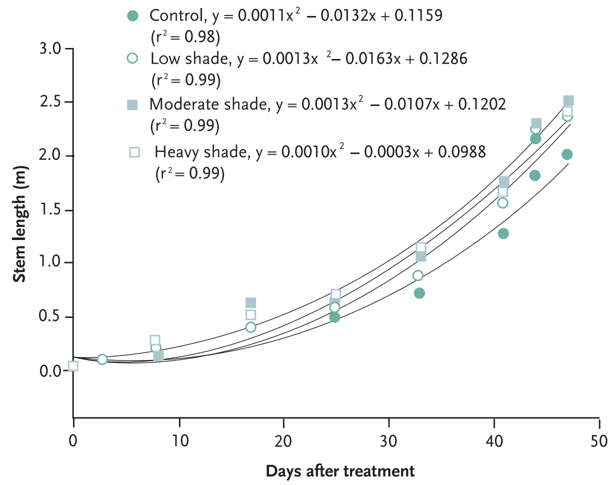

Figure 5. Total stem lengths of plants grown under full sun (control), or low (30%), moderate (60%) or heavy (≈90%) shade as a function of time.

Institute Inc, Cary, North Carolina), and to regression analysis, using the regression procedure of SAS, or regression functions of Sigma Plot (SPSS Inc, Chicago, Illinois). When treatment effects were significant ($\alpha = 0.05$) treatment means were separated by Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

Stem length of *J. reclinata* seedlings was a quadratic function of time for the duration of the experiment; growth was slow when the seedlings were small but increased markedly as seedling size increased, regardless of shade treatment (Figure 5). Such increasing shoot extension rates are typical of seedlings and young vegetative shoots (Chiariello and others 1994). Shade did not affect the rate of shoot extension (Figure 5), so plants of all treatments had similar total stem length at harvest (data not shown). Likewise, leaf number, leaf dry weight, and stem dry weight (data not shown), and total shoots (leaf + stem) dry weight (Table 1) were similar for all treatments. In contrast, the shoots of *J. tamnifolia* plants grown under 30% to 90% shade for 7 wk were 38% to 87% smaller than the shoots of non-shaded plants (Shaw and others 1987). This suggests that *J. reclinata* has greater shade tolerance than *J. tamnifolia.*

In contrast to shoot mass, root mass of *J. reclinata* was reduced dramatically by shade; seedlings grown under any level of shade amassed only 40% to 70% of the root mass of non-shaded plants (Table 1). Therefore, the root-to-shoot ratio of shaded plants was 40% to 50% less than that of non-shaded plants. Plants with large root systems may be more resistant to environmental stress, especially water deficit stress, than those with smaller root systems because their expansive roots may explore a greater volume of soil for water. These large root systems could be beneficial to plants growing in dry, warm conditions in which roots need to be efficient water and nutrient collectors (Carter 1988; Jenkins 1999). Shade might benefit seedlings in nature, however, by creating a microclimate where temperature and vapor pressure deficits are more moderate and plant water use is less. For plants growing in containers, however, high root-to-shoot ratios can be disadvantageous because, in the restricted rooting volume of containers, plants with large root systems may deplete available water sooner than plants with smaller root systems (Levy and others 1983; Fidelibus and others 2001).

Despite the dramatic effect of shade treatments on root mass, treatments did not affect total plant dry mass (Table 1). Shade effects on plant mass probably were not detected in this experiment because seedling variability was high relative to the number of replicates. Moreover, seedlings had relatively low root-to-shoot ratios (0.23 to 0.48), so treatment effects on root mass had little effect on total plant dry mass. Total dry mass, however, was positively correlated with root mass ($r^2 = 0.61$); thus, shade may eventually limit plant growth.

Regardless of shade treatment, plants in this experiment had 10X to 20X greater dry mass than 4-mo-old *J. reclinata* seedlings grown in a glasshouse (70% light exclusion) with less fertilizer (Fisher and Jayachandran 2002). Thus, *J. reclinata* seedlings are highly responsive to fertilization, even under heavy shade. Moreover, one of the non-shaded plants in this study flowered within 47 d, indicating that the plants' juvenility period might be reduced by optimal cultural conditions; a finding that could be utilized when growing plants for seed.

Fructose concentrations in *J. reclinata* roots were at or below the level of detection (0.2%), so those data were not analyzed. Glucose levels of roots were also low, and shade did not affect glucose concentrations (Table 2). The dominant non-structural carbohydrate of *J. reclinata* roots was sucrose;

TABLE 1

Shoot and root dry mass, root-to-shoot ratio, and total plant dry mass of Jacquemontia reclinata *grown for 47 d under full sun (control), or low (30%), moderate (60%), or heavy (≈90%) shade.*

Treatment	Shoot dry mass (g)	Root dry mass (g)	Root:shoot (g:g)	Total dry mass (g)
Control	2.20[a] a[b]	1.04 a	0.48 a	3.25 a
Low	2.41 a	0.66 b	0.28 b	3.07 a
Moderate	2.17 a	0.67 b	0.30 b	2.83 a
Heavy	1.76 a	0.40 b	0.23 b	2.16 a

[a] Values are treatment means, n = 3.

[b] Means followed by a different letter are significantly different (α= 0.05) according to Duncan's New Multiple Range Test.

TABLE 2

Concentration and content of glucose, sucrose, and starch in roots of Jacquemontia reclinata *grown for 47 d under full sun (control) or low (30%), moderate (60%), or heavy (≈90%) shade.*

	Glucose		Sucrose		Starch	
Treatment	% dry weight	Content (g/root $^{-1}$)	% dry weight	Content (g/root^{-1})	% dry weight	Content (g/root^{-1})
Control	0.30[a] a[b]	0.0031 a	17 a	0.17 a	2.8 a	0.030 a
Low	0.37 a	0.0022 ab	12 ab	0.08 b	2.5 ab	0.017 b
Moderate	0.27 a	0.0018 ab	9 b	0.06 b	2.2 b	0.015 b
Heavy	0.23 a	0.0010 b	9 b	0.04	2.3 b	0.009 b

[a] Values are treatment means, n = 3.

[b] Means followed by a different letter are significantly different (α = 0.05) according to Duncan's New Multiple Range Test.

roots contained 4X to 8X more sucrose (on a concentration basis) than starch. Sucrose concentrations were much higher in the roots of plants grown in full sun compared to those grown in shade; the roots of plants grown under moderate or heavy shade had only about half the root sucrose concentration of non-shaded plants. Root starch concentrations were also lower in plants grown under moderate or heavy shade compared to the roots of plants that were non-shaded.

Because non-shaded plants had greater root weights than shaded plants, the content of soluble sugars and starch was affected more than their concentrations (Table 2). For example, the roots of non-shaded plants amassed 3X more glucose than the roots of plants grown in heavy shade. Likewise, non-shaded plants had 2X to 4X more sucrose and 2X to 3X more starch than shaded plants.

When nutrients and water are non-limiting, as in this experiment, plants may be expected to allocate more resources to storage organs, such as roots, when photosynthates are plentiful, and to shoots when photosynthates are limited (Koch 1996). Thus, the smaller root mass and less carbohydrate reserves of plants grown in the shade suggests that shade limited photosynthesis of *J. reclinata* seedlings. Shade may affect the plants differently, however, if other resources, such as nutrition or water, are limiting. For example, plants that received little fertilizer and were grown in a glasshouse had larger root-to-shoot ratios than those observed in our study (Fisher and Jayachandran 2002).

Jacquemontia reclinata plants rely on their root systems to regenerate shoots following fire and herbivory (Robertson 1971). Therefore, our data suggest that restoration and conservation practices that reduce shade may benefit *J. reclinata* because non-shaded plants should develop larger root systems with greater carbohydrate reserves and thus be more resistant to stress and disturbance than plants grown in shade. This experiment was conducted between June and July because *J. reclinata* generally flowers from November to May (USFWS 1999), and seeds set during this time might be expected to germinate and establish in the summer rainy season. Southeast Florida typically has periodically cloudy skies during the summer, so non-shaded plants were rarely exposed to "full-sun." Thus, similar shade treatments might affect *J. reclinata* differently if applied in the spring or fall when skies are less cloudy (Fisher and Jayachandran 2002).

In summary, under conditions of non-limiting soil fertility and moisture, shade decreased root growth and limited carbohydrate reserves of *J. reclinata* seedlings. Non-shaded plants accumulated nearly twice as much root dry mass and more than 2X to 4X as much non-structural carbohydrates as plants grown in shade. Therefore, beneficial effects of shade, if any, are probably not related to light levels per se, but might be related to other factors such as lower vapor pressure deficits or higher soil moisture availability. We recommend areas of partial (< 30% light exclusion) to full sun be utilized for locations of reintroduction projects, and the openness of the coastal strand maintained.

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