

REPRODUCTIVE POTENTIAL, GROWTH RATE AND LIGHT ENVIRONMENT IN *LEPANTHES RUPESTRIS* STIMSON

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Reproductive success in orchids can be pollinator or resource limited (Ackerman & Montalvo 1990). However, orchids are generally pollinator limited (Neiland & Wilcock 1998), while some species have shown to be resource limited as a function of lifetime reproductive success (Whigham & O'Neill 1991, Meléndez-Ackerman *et al.* 2000). Large individuals within a species usually have more flowers, and flower production is frequently correlated with male (pollinaria removal) and female (fruit) reproductive success (Schemske 1980; Montalvo & Ackerman 1987; Aragón & Ackerman 2001, Kull 2002, Schmidt & Zotz 2002). Plant size can affect flower production and accordingly potential reproductive success (Schaffer 1974, Samson & Werk 1986, Kull 2002). Consequently, environmental conditions that modify plant size will likely influence reproductive effort and success. Even though reproductive success in orchids is primarily pollinator limited, individuals with more flowers have a higher probability of male or female reproductive success (Schemske 1980). This pattern may suggest that pollinators focus on inflorescence size (Montalvo & Ackerman 1987; Rodríguez-Robles *et al.* 1992, Aragón & Ackerman 2001, but see Sabat & Ackerman 1996). Zotz (2000) found that the number of fruits produced ($r^2 = 0.74$) and total fruit mass ($r^2 = 0.72$) were positively correlated with plant size in the facultatively self-pollinated *Dimerandra emarginata* (G. Meyer) Hoehne. Moreover, Zotz (2000) found that the smallest plants invested less than 1% of the annual proportion of the biomass to reproduction while the larger plants invested in the range of 12% to reproduction. However, flower production does not necessarily respond linearly to light availability; of the few examples available, *Cypripedium calceolus* is light sensitive, and the relationship between percent flowering shoots

and light penetration coefficient is a quadratic function (Kull 2002). Growth rate in plants is frequently site/year dependent and local conditions can ultimately influence reproductive potential (Schmidt & Zotz 2002). The present information on growth rates of orchids in natural environments and controlled conditions is scarce (Zotz 1999, Schmidt & Zotz 2002, Zimmerman & Aide 1989). Schmidt & Zotz (2002) showed that growth rates in *Aspasia principissa* Rchb.f. was different among *in situ* and greenhouse grown plants, but more or less similar among years. The Neotropical genus *Lepanthes* is a large group of epiphytic and lithophytic orchids growing in a variety of environmental habitats, from complete exposure to very low understory light. For this study we proposed to investigate: 1) the effect of total leaf area on flower production in a controlled setting, 2) the relationship between total flower production and reproductive success in the field, 3) the light environment of the populations, and 4) the effect of light quantity on growth rates of the individuals in natural populations of *Lepanthes rupestris*.

Plant species. We evaluated the reproductive potential, growth, and the photosynthetic radiation niche requirement of the epiphytic and lithophytic orchid *L. rupestris*, an endemic of Puerto Rico. The species is mainly limited to the Caribbean National Forest in the subtropical moist forest (Ewel & Whitmore 1973) and is common along rivers on boulders, palms and trees in a riparian environment. The species is hyperdispersed, with many small populations and few large populations (median, mean and s.e. 23; 45.4 ± 5.2 individuals per populations) and these are separated by variable distances, but most frequently nearby (mean and s.e. 4.8 ± 1.3 m., Tremblay 1997).

Laboratory experiment; Leaf area and flower production. Two hypotheses were tested with this experiment: can total leaf area and number of leaves per individual predict the long-term flower production? Fourteen individuals of *L. rupestris* were grown in a Wardian case (Orchidarium Inc.) for eight months under growth lights for 14 hours/day. Environmental conditions were held constant with a mean temperature of 23 °C and a 95% relative humidity. Plants were watered when necessary (every two to four days) with distilled water and fertilized every two weeks with a 20-20-20 solution (P-K-N: half a teaspoon per liter; Tropical Fertilizer Corp., Puerto Rico). Data were collected weekly, and all flower production was counted. Total leaf area (A) produced was calculated using a caliper to measure the width and length of each leaf and applying the following formula: $A = 1.51 + 0.57b$, where $b = \text{length} \times \text{width}$. Leaf area was pooled for each individual. Linear and quadratic regressions were used to test the relationship between number of leaves and leaf area with flower production (StatView, Inc., Abacus Concept Inc., California.). Analysis was performed on the square root of number of flowers and leaf number and on the log transformed leaf area to reduce heterocedasticity.

Field observation I: Flower production. Male and female reproductive success in a population of 98 individuals of *Lepanthes rupestris* at Quebrada Grande, Luquillo Mountains, was monitored from July 1993 for a total of 21 months. Plants were surveyed every month; flowers have a survivorship of approximately 1.5 weeks while fruits last about 1.5 months on the plant. Consequently approximately 1/3 of all flowers produced were observed for pollinia removal while all fruits during the period were noted. The relationship between flower production and pollinaria removal and fruit set was analyzed using Spearman Rank correlation (StatView.)

Field observation II: Diversity of the light environment and its effect on leaf area production. How variable is the light environment between populations, and how is the temporal distribution of the photosynthetic radiation during the day of the different populations? We tested whether or not variable light environment measured as the daily total radiation affected the production and growth of leaves in individual populations. This field study was conducted at El Verde Field Station in the Luquillo Mountains, a few km from Quebrada Grande. The

populations under study were along Quebrada Sonadora. Twelve sites with one or more populations of *L. rupestris* were located for this study. The sites represent the range of light environments where the orchids are found and these were classified according to the canopy cover, closed (canopy cover above 90 %), medium (between 60 and 90 %), and open (below 60 %). We assume that light was equal for all plants at an individual site. The light environment of each population was measured as photon flux density (PFD 400-700 nm, $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). The photon flux density is the component of the solar spectrum which is most related to photosynthetic activity. We measured PFD with previously calibrated GaAsP photodiodes (G1118, Hamamatsu, Japan); the sensors were connected to a data logger (CR-10, Campbell Scientific, Utah) programmed to take measurements every second, and to total and store data every five minutes, between 6:00 and 18:00 hours of solar time. With the data we calculated instantaneous average values of PFD every five minutes and total daily values (mol m^{-2}). We measured the changes in leaf area that occurred between June 2001 and August 2001. Eleven plants from each of the 12 sites were selected. We expected that too little light would result in poor growth while growth rate should improve and attain a plateau or maximum with increased light. Consequently, we could not assume a specific reaction model of light quantity and growth (linear, quadratic) so we used a non-parametric analysis to study the selection response of light quantity and growth rates. We used the cubic spline technique to graph the reaction response function and its variance (Schluter & Nychka 1994).

RESULTS

Flower production and reproductive success. The total flowers produced by individual plants in the field varied from 4 to 269, (mean and s.d. = 63.6 ± 59.8), while the number of pollinaria removal varied from 0 to 23 (mean and s.d. = 3.03 ± 4.01) and the number of fruits varied from 0 to 13 (mean and s.d. = 1.93 ± 2.52). In all cases variation in reproductive potential among individuals was large. Male reproductive success was positively correlated with flower production (simple linear regression, $F_{1,97} = 83.50$, $p < 0.0001$. $r^2 = 0.46$: Fig. 2). While the number of flowers produced was also positively correlated with female reproductive success it explained more of the

variation than male reproductive success (simple linear regression, $F_{1,97} = 142.65$, $p < 0.0001$, $r^2 = 0.60$).

Correlation between leaf area and flower production. Flower production is positively and linearly correlated with the leaf area of individuals in the laboratory (linear regression $F_{1,11} = 24.91$, $p < 0.001$, $r^2 = 0.69$; square root of number of flowers = $-7.436 + 4.409 * \text{logarithm of leaf area}$). The quadratic equation explained more of the variance, $r^2 = 0.80$, but was not significant ($t\text{-value} = -2.126$, $p = 0.060$).

Correlation between leaf number and flower production. Flower production is directly correlated with the number of leaves per individual (linear regression $F_{1,11} = 7.017$, $p = 0.022$, $r^2 = 0.39$; square root of number of flowers = $0.687 + 1.937 * \text{square root of number of leaves}$). The quadratic equation explained more of the variance, $r^2 = 0.58$, than the linear equation but was not significant ($t\text{-value} = -2.141$, $p = 0.058$).

Description of total PFD received by differing light cover of *Lepanthes rupestris* population. The amount of light received by populations of *Lepanthes* is expected to vary as a result of canopy cover over the population. The amount of light was significantly different among the three site types. Open canopy populations received more than twice the amount of light as compared to medium covered populations ($6.77 \pm 2.41 \text{ mol m}^{-2}$ and $2.89 \pm 0.58 \text{ mol m}^{-2}$, mean and s.e.), while closed canopy barely received any light (0.303 ± 0.063 mean and s.e.). Furthermore the amount of light was significantly higher in the afternoon (mean and s.d.: AM: $1.21 \pm 1.21 \text{ mol m}^{-2}$; PM: $2.62 \pm 3.74 \text{ mol m}^{-2}$); however, no interaction was observed between canopy cover and time of day.

Light quantity and growth rates. The cubic spline analysis of correlation between amount of light and growth rate was non-linear and suggested that increasing growth rates occurred at irradiation ranging from 1 to 5 $\text{mol m}^{-2} \text{ day}^{-1}$, while higher total daily PFD resulted in reduced growth rates. The data points are scattered below and above the best non-parametric fitness line suggesting that other environmental variables are likely to influence growth. The squiggled pattern observed was similar when the analyses were done with the rock and tree populations separately.

DISCUSSION AND CONCLUSIONS

Field observations showed that an increase in flower production promotes a higher reproductive potential, with both male and female reproductive success correlated with flower production. Flower production measured as inflorescence size has shown to positively correlate with reproductive potential in a number of orchids, such as higher fruit set in *Brassavola nodosa* (Schemske 1980), *Lepanthes wendlandii* (Calvo 1990), *Calopogon tuberosus* (Firmage & Cole 1988), *Ionopsis utricularioides* (Montalvo & Ackerman 1997) and *Aspasia principis* - *sa* (Zimmerman & Aide 1989). However some species of orchids have failed to show an increase in fruit set with increased flower production, v.g. *Psychilis krugii* (Ackerman 1989) and *Epidendrum exasperatum* (Calvo 1990). Inflorescences of *Lepanthes* are long lived and most of the time only one or rarely two flowers are open, this different strategy of flowering (sequential vs. synchronous) has been shown to improve male and female reproductive success in *Psychilis monensis* (S. Aragón, unpublished).

As in most plants, plant size distribution was not normally distributed but skewed towards small plants (Weiner & Solbrig 1984, Gregg 1991, Leeson, Haynes & Wells 1991). We would thus expect that flower production be skewed towards few flowers per plant. The controlled conditions experiment showed that the flower production of *L. rupestris* is positively related with the area of the photosynthetic tissue, that is, with the amount of leaf area and the number of leaves, which are also correlated among them. *Lepanthes rupestris* shows a large variation in flower production per individual, thus the factors that control plant size will also limit the flower production.

What limits plant size in natural environment is still poorly studied in orchid in general. An interesting example is shown in *Catasetum viridiflavum* where plant size is dependent on availability of resources and light environment. Plants fully exposed to light are more likely to be large and female, while plants found in the shade are small and produce male flowers; however, plants in a resource rich environment with limited amount of light could be large and female (Zimmerman 1990, 1991). The range of the photosynthetic light environment where *L. rupestris* is found is very broad in terms of the daily totals, and the distribution of PFD through the day is not uni-

form due to the topography and aspect of the area. *Lepanthes rupestris* needs low values of daily total PFD to attain maximum growth in terms of leaf area production. The negative effect on growth of daily total PFD values above 9 moles m⁻² could result from a combination of water stress due to dessication of the microenvironment, and chronic photoinhibition.

In conclusion, reproductive success in *L. rupestris* is directly related with flower production and flower production is directly related to plant size, but the relationship between vegetative growth and the photosynthetic light environment is less evident, probably because it is mediated by other factors that affect orchid physiology, especially carbon gain.

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