

— Short Communication —

PRODUCTION OF FLORAL MORPHS IN CLEISTOGAMOUS *RUELLIA BREVIFOLIA* (POHL) C. EZCURRA (ACANTHACEAE) AT DIFFERENT LEVELS OF WATER AVAILABILITY

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Abstract—In this study we investigated whether the production of cleistogamous (CL) and chasmogamous (CH) floral morphs in *Ruellia brevifolia* is affected by water availability. To this end, the effects of two water levels were tested on plants grown in a greenhouse: soil at 100% water-holding capacity (WHC) (moist soil) and at 50% WHC (water scarcity). Additionally, we investigated fruit and seed production in plants at these two levels of water availability and evaluated whether the drought stress interferes with vegetative growth. The production of floral morphs depended on water availability: plants in moist soil produced only CH morphs and water-stressed plants produced only CL morphs. Fruit production was higher at the higher level of water availability (30.5 ± 28.20 fruits/plant at 100% WHC versus 9 ± 6.04 fruits/plant at 50% WHC; $t = 4.384$; $P < 0.01$). The mean number of seeds produced by CH and CL morphs were, respectively, 5.93 ± 2.24 and 8.17 ± 2.07 seeds/fruit ($t = -3.304$; $P < 0.01$). Although CL morphs produced a greater number of seeds, the total seed production per plant was higher in plants at 100% WHC (180.86 seeds/plant in CH morphs versus 73.53 seeds/plant in CL morphs of plants in soil at 100% and 50% WHC, respectively; $t = -2.759$; $P < 0.01$). The plants in soil at 100% WHC were taller ($0.48 \text{ m} \pm 0.07$) in relation to plants in soil at 50% WHC ($0.24 \text{ m} \pm 0.04$) ($t = 1.781$; $P < 0.01$). This study provides new information about the sexual reproductive strategy of *R. brevifolia*, indicating that the main factor inducing cleistogamy is drought stress.

Keywords: chasmogamous floral morph, cleistogamous floral morph, drought stress, floral induction, floral polymorphism, reproductive systems

INTRODUCTION

Ruellia is the largest genus of Acanthaceae, with about 300 species distributed in the tropics (Ezcurra 1993; Tripp 2007). Three species occur at the study location in Viçosa, Minas Gerais, southeastern Brazil (Braz et al. 2002). Two of them, *Ruellia brevifolia* and *Ruellia menthoides*, are typically cleistogamous (Lima et al. 2005; Lima & Vieira 2006), i.e., they produce cleistogamous (CL) and chasmogamous (CH) floral morphs. For the third, *R. subsessilis*, typical cleistogamy has not been reported. This species produces, instead, two distinct chasmogamous morphs ("reduced" and "normal" chasmogamous morphs), depending on the availability of soil moisture (Miranda & Vieira 2014).

Ruellia brevifolia is ornithophilous (bird pollinated; Piovano et al. 1995; Braz et al. 2000; Sigrist & Sazima 2002; Abreu & Vieira 2004), herbaceous, and about 1.0 m tall. The inflorescences are axillary and bear open, potentially

outcrossed (CH), red flowers, and closed, obligately self-pollinated (CL), white flowers (Piovano et al. 1995; Sigrist & Sazima 2002; Lima et al. 2005). The pollination tests with the CH morph, performed by Lima & Vieira (2006), obtained similar fruit set without (autofertility, 42.10%) and with pollinators (fruit set of open-pollinated, emasculated flowers, 44.74%). In Viçosa, *R. brevifolia* is found in the forest understory, in shady to partially shaded locations, with year-round flowering and fruiting (Lima et al. 2005; Lima & Vieira 2006).

Observations of Lima et al. (2005) suggest that CH and CL morphs of *R. brevifolia* are produced throughout the year, except in August and September (dry season) for the CH morph and except for January and February (rainy season) for the CL morph. The alternation of chasmogamous and cleistogamous cycles in *R. brevifolia* has been related to ecological and climatic conditions, because the production of CL morphs was mainly observed in the months of low precipitation and temperature (Piovano et al. 1995; Sigrist & Sazima 2002). It is generally thought that the allocation of resources for the production of each floral morph may be influenced by water availability (Lord 1981),

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and by variations in the photoperiod (Langer & Wilson 1965), temperature (Hexslo 1888), and light intensity (Schemske 1978). However, only a few studies have assessed the role of such factors in *Ruellia* (e.g. *Ruellia nudiflora*, Munguías-Rosas et al. 2012) and or other species (e.g. *Impatiens* sp., Schemske 1978; *Collomia grandiflora*, Minter & Lord 1983; *Calathea micans*, Le Corff 1993).

This study assessed the production of floral morphs in *R. brevifolia* at two levels of water availability in plants grown in a greenhouse: soil at 100% water-holding capacity (WHC) (moist soil) and at 50% WHC (water scarcity). In addition, fruit and seed production in plants at these two levels of water availability and the influence of drought stress on vegetative growth were investigated.

MATERIALS AND METHODS

The experiments were conducted from May 2009 to January 2010 in a greenhouse of the Federal University of Viçosa. *Ruellia brevifolia* plants ($N = 20$) were randomly obtained from previously rooted cuttings (about 25 cm long) from adult plants of a natural population in Viçosa (with about 100 plants), at the Station of Research, Environmental Training and Education Mata do Paraíso, a semideciduous forest reserve. The climate of Viçosa is characterized by hot, humid summers from October to March and cool, dry winters from April to September (Pezzopane 2001).

The plants were grown in pots filled with 3 Kg of substrate consisting of a mixture of soil from the site of plant occurrence with sand (ratio 2:1). The preliminary chemical analysis of this substrate indicated suitability for cultivation. About 50 days after planting (plants in reproductive stage), watering treatments were applied to soil with: 100% of water-holding capacity (WHC) (moist soil, control; $N = 10$ plants) and 50% WHC (drought stress; $N = 10$ plants).

The water-holding capacity was determined in five 100 g samples of the dried substrate at 103 °C for 48 hours, by saturation with water until the percolated water volume became constant (Freire et al. 1980). The values were extrapolated to the amount of soil contained in the pots, corresponding to the control with 100% WHC, and a value of 50% WHC was determined. Irrigation was monitored by the gravimetric method (weighing the pots), adding water until the pots reached the predetermined value for water-holding capacity, based on soil and water weight (Freire et al. 1980).

The 20 plants were checked weekly for the presence of CH and CL morphs. The average numbers of fruits per plant of both water treatments were calculated on two occasions: in August (two months after treatment) and in December 2009 (six months after treatment). In addition, the average number of seeds per fruit ($N = 30$, 15 per treatment) was calculated in December 2009.

To ensure that the plants were drought-stressed (50% WHC), three plants of each treatment (drought stress and control) were evaluated in January 2010, between 8:00 and

10:30 AM, for net photosynthesis (A), stomatal conductance (G_s) and transpiration rates (E). For this purpose, we used an infrared gas analyzer (Irga) - Licor 6400, with steady light sources $700 \mu\text{mol m}^{-2} \text{s}^{-1}$, indicated as optimum value by the light curve.

The plant height (base to stem apex) was measured at the end of the experiment, to compare the vegetative growth of the plants under water stress compared to those in moist soil ($N = 20$, 10 per treatment). From each value, the initial length of the cuttings was subtracted.

The data were tested for normality and homogeneity (Kolmogorov-Smirnov and Cochran C) and when normal and homogeneous, the Student test was used (Zar 1996).

RESULTS AND DISCUSSION

Plants grown in soil at 100% WHC produced only CH morph and plants in soil at 50% WHC only CL morphs. According to Brown (1952), drought stress may be the trigger of cleistogamy. Indeed, in this study, the monthly means of soil water potential (ψ_w) estimated during the experiment (100% WHC: $\psi_w = 0.15$ MPa; 50% WHC: $\psi_w = -0.36$ MPa) showed that the water scarcity treatment probably induced drought stress. Moreover, the net photosynthesis (A ; $\mu\text{mol m}^{-2} \text{s}^{-1}$), transpiration (E ; $\text{mmol m}^{-2} \text{s}^{-1}$) and stomatal conductance rates (G_s ; $\text{mol m}^{-2} \text{s}^{-1}$) were higher in plants grown in soil at 100% WHC ($A = 4.8$; $E = 0.73$ and $G_s = 0.043$) than in plants grown in soil at 50% WHC ($A = 0.06$; $E = 0.15$ and $G_s = 0.008$).

For these reasons, the gene expression of cleistogamy seems to have been selected in stressed plants, possibly due to the lower production and transpiration costs for CL morphs (Galen et al. 1999; Webster & Grey 2008), which are smaller and produce no floral nectar, as seems to be the case of the studied species. Based on this premise, the production of different floral morphs in natural *R. brevifolia* populations should have a seasonal pattern, in response to the water level throughout the year. Thus, plants tend to produce CH morphs in the period of greatest soil water availability (rainy season), and CL morphs in the period of lower availability (dry season). The findings of Sigris & Sazima (2002) and Lima et al. (2005) for this species demonstrated this trend, reinforcing the results of this study.

Fruit production was higher at the higher level of water availability (30.5 ± 28.20 fruits/plant at 100% WHC versus 9 ± 6.04 fruits/plant at 50% WHC; $t = 4.384$; $P < 0.01$). During the experiment, the fruit set of plants grown in soil at 100% WHC decreased (from 50 ± 27.34 fruits/plant in August to 11 ± 8.02 fruits/plant in December; $t = 4.384$; $P < 0.01$), but no significant variation was observed in plants on 50% WHC soil (from 11 ± 6.92 fruits/plant in August to 6.7 ± 4.4 fruits/plant in December).

The reduction in fruit production throughout the experiment in plants at 100% WHC may result from changes in the photoperiod (Langer & Wilson 1965), temperature (Hexslo 1888), light intensity (Schemske

1978), or also from nutrient depletion in the potting soil. Additional studies are needed to confirm these possibilities.

The lower fruit set of plants at 50% WHC may be explained by the reduced photo assimilation, which could reduce the amount of assimilates allocated to fruit production (Garrido et al. 2000). These plants may be resilient to this stress level, since fruiting throughout the experiment varied little, as similarly observed in *R. subsessilis* by Miranda & Vieira (2014).

The average numbers of seeds produced by CH and CL morphs were, respectively, 5.93 ± 2.24 and 8.17 ± 2.07 seeds/fruit ($t = -3.304$; $P < 0.01$). The higher seed production by CL morphs was due to the more efficient self-pollination process (95% of fruit set, Lima & Vieira 2006). The smaller number of seeds of CH morphs resulted from self-pollination that was less efficient (42.10% of fruit set, Lima & Vieira 2006). Despite the greater per-flower number of seeds produced by CL morphs (only in plants on 50% WHC soil), the total seed production per plant was higher in CH-morph plants on 100% WHC soil (180.86 ± 167.31 seeds/plant in plants at 100% WHC versus 73.53 ± 49.33 seeds/plant in plants at 50% WHC; $t = -2.759$; $P < 0.01$), showing that drought stress can reduce the reproductive success of *R. brevifolia*.

The plants in 100% WHC soil were taller than plants in 50% WHC soil (respectively, $0.48 \text{ m} \pm 0.07$ and $0.24 \text{ m} \pm 0.04$; $t = 1.781$; $P < 0.01$), because the water stress affected vegetative growth, as previously reported (Larcher 2004). Changes in soil moisture can also alter the availability of nutrients such as nitrogen (Birch 1964), which can also affect plant growth in soil at 50% WHC.

Conclusions

Our greenhouse experiments demonstrated that water availability is a primary factor in the induction of the type of floral morph produced by in *R. brevifolia*: CH morphs are produced in high-moisture soil (100% water-holding capacity) and CL morphs are produced by plants in drier soil (50% WHC). Drought stress reduces vegetative growth and fruit and seed production. All three measures were higher in plants on 100% WHC soil, which produced only CH flowers.

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