UNIVERSIDADE FEDERAL DE LAVRAS

GUILHERME RAMOS DEMETRIO FERREIRA

CLONAL INTEGRATION INFLUENCES ON LIFE-HISTORY TRAITS OF *EICCHORNIA CRASSIPES* (MART.) SÖLMS. (PONTEDERIACEAE)

LAVRAS-MG
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Profª. Drª. Flávia de Freitas Coelho

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INFLÛENCIAS DA INTEGRAÇÃO CLONAL EM ASPECTOS DA HISTÓRIA DE VIDA DE EICCHORNIA CRASSIPES (MART.) SÖLMS. (PONTEDERIACEAE)

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APROVADA em 21 de Julho de 2017.

Profª. Drª Tatiana Garabini Cornelissen

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LAVRAS-MG
2017

A minha mãe, pelo ensinamento de que o bom humor é a chave.

A meu pai, pela certeza de que o tijolo do sucesso é o conhecimento

Dedico

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“There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved.”

— **Charles Darwin**, **The Origin of Species**

RESUMO

A clonalidade é um traço chave na história de vida das plantas e através da integração clonal entre rametes mãe e filhos podem haver alterações nos padrões de investimento de recursos. O objetivo deste trabalho foi verificar a influência da clonalidade, especificamente observada a integração clonal, em aspectos de história de vida de *Eicchornia crassipes* (Mart.) Sölms. A partir de experimentos realizados em casa de vegetação, investigamos a relação dos traços florais, da variação intra-individual em traços florais e dos padrões de alocação de biomassa com a manutenção da conexão entre rametes originados via reprodução assexuada e seus rametes maternais. Para isso, coletamos plantas adultas na Represa do Funil, em Ijaci, cuja segunda geração assexuada em casa de vegetação foi utilizada para o experimento. Durante três meses os indivíduos foram avaliados diariamente para verificação da floração e medição dos caracteres florais. Ao final do experimento, os indivíduos foram levados ao laboratório e suas partes (caule, limbo foliar, pecíolo e raízes) foram separadas, passaram por secagem em estufa e foram pesadas. A partir da análise desses dados, encontramos que plantas clonais, em geral, apresentam flores maiores e mais robustas. Além disso, os traços florais variam menos, em nível intra individual, nas plantas clonais do que nas plantas isoladas, indicando que a integração clonal garante o repasse de recursos para os rametes filhos em casos de baixa disponibilidade ambiental. A partir desses resultados, concluímos que a clonalidade representa um mecanismo que proporciona aumento da capacidade do investimento em reprodução sexuada e que está possivelmente ligada à garantia da polinização e da geração de propágulos viáveis em diferentes situações ambientais.

Palavras-chave: Alocação de biomassa. História de vida. Modos reprodutivos.

ABSTRACT

This work aimed to examine how clonality, specifically clonal integration, influences the life history traits of *Eicchornia crassipes* (Mart.) Sölms. We developed greenhouse experiments in order to comprehend the relationship between floral traits, intra-individual variation on floral traits and patterns of biomass allocation with the maintenance between ramets generated by asexual reproduction and mother ramets. We collected adult individuals on Represa do Funil, Ijaci, and used their second asexual offspring generation to carry out the experiments. Individuals were daily observed during three months, and floral traits were sampled. At the end of the experiment plants were taken to the laboratory, where they were separated into parts (stems, leaf blades, petioles and roots), dried, and weighed. We found that clonal ramets exhibited larger and more robust flowers. In addition, the floral traits were less variable, on the intra-individual level, on clonal ramets than on isolated ones, indicating that clonal integration guarantee the transport of resources from mother to daughter ramets in cases of low environmental availability. These results lead us to the conclusion that clonality plays a central role on water hyacinth life history, ensuring an increase on sexual reproduction investment and is possibly linked to a viable production of propagules in different environmental conditions.

Keywords: Biomass allocation. Life history theory. Reproductive modes.

Sumário

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

As plantas são organismos altamente plásticos (SCHLICHTING, 1986; GIVINISH, 2002; BORGES, 2005, 2008). Essa alta plasticidade fenotípica permite que um genoma modifique, por exemplo, seus padrões de crescimento e desenvolvimento em resposta às mudanças ambientais (DORKEN; BARRET, 2004), principalmente pela constituição modular do corpo desses organismos (HARPER, 1977, 1980). Grande parte dessa plasticidade está ligada às estratégias reprodutivas, já que a maior parte das plantas possui a habilidade de gerar propágulos sexuados e assexuados (HARPER, 1977).

A relação entre os modos reprodutivos e as condições ambientais em que se encontra um indivíduo é uma questão largamente debatida na ecologia vegetal e estaria ligada tanto a questões ambientais (HARPER, 1977; ABRAHAMSOM, 1980; COOK, 1985; COELHO et al., 2000; COELHO et al., 2005), quanto a questões intrínsecas à planta, tais como o tamanho de corpo (ISHII; MORINAGA, 2005; WEINER et al., 2004, 2009).

Um grande corpo teórico tem se acumulado em torno desta linha de pesquisa e, em geral, tem-se suportado a existência de *trade-offs* (STEARNS, 1992; ROFF, 2002) entre esses modos reprodutivos (GRACE, 1993; COELHO et al., 2000), e esses *trade-offs* podem significar uma forma de garantia da reprodução do indivíduo (WESTLEY, 1993).

Essa discussão se projeta como um tópico de alta importância para a ecologia das macrófitas aquáticas, o grupo de plantas vasculares com maior diversidade de sistemas reprodutivos (BARRET, 1993). No caso deste grupo, a reprodução assexuada parece ser altamente importante (BARRET, 1980; WATSON; COOK, 1987; BARRET, 1993). Essa predominância de reprodução assexuada pode estar ligada à alta probabilidade de falha da reprodução sexuada em ambientes aquáticos (SCULTHORPE, 1967; PHILBRICK; LES, 1996) e a uma maior capacidade de ocupação de habitat quando as condições ambientais são favoráveis (COELHO et al., 2000; COELHO et al., 2005). A predominância da reprodução assexuada nas plantas aquáticas também parece ser fruto de um efeito filogenético. Isso se sustenta pela existência de um forte viés para a presença da clonalidade nas Monocotiledôneas, quando comparadas às Eudicotiledôneas (GRACE, 1993), que estaria ligado à ausência de um câmbio vascular naquele grupo (TIFFNEY; NIKLAS, 1985) um facilitador da expansão lateral.

A clonalidade tem efeitos diretos dos modos reprodutivos na biologia de populações das plantas, apresentando diferentes níveis de sucesso de propágulos (GRACE, 1993; COELHO et al., 2008a). Além disso, também está relacionada à muitos outros processos ecológicos nas plantas clonais como a colonização de diferentes habitats (COELHO et al., 2008b; DEMETRIO; COELHO, 2017) e à capacidade de reprodução sexuada por ramete (DEMETRIO et al., 2014). Dessa forma, espera-se, inclusive, que diferentes formas de reprodução clonal tenham diferentes papéis nas estratégias de história de vida das plantas (GRACE, 1993; XIE et al., 2016).

Um dos principais fatores que condiciona essa ampla capacidade de respostas é, provavelmente, a integração fisiológica entre as plantas mãe e filhas, também chamada de integração clonal, que permite uma divisão de funções dentro de um clone, com cada ramete apresentando processos específicos como aquisições de recursos ou reprodução sexuada (STUEFER et al., 1994; WANG et al., 2011). No caso da relação entre reprodução sexuada e assexuada, essa ligação entre rametes mãe e filho pode ter um efeito que difere do *trade-off* esperado, no qual um aumento na reprodução sexuada causaria um decréscimo na reprodução assexuada, principalmente porque os órgãos e a prole clonal também podem funcionar como centrais de aquisição de recursos (PHILBRICK; LES, 1996; BAZZAZ, 1997; Demetrio et al., 2014; GUO et al., 2017). Assim, a integração clonal pode favorecer o conjunto de rametes mãe e filhos como um todo (ALPERT; SIMMS, 2002).

Até o presente momento, a maior parte dos trabalhos que analisa a relação entre as biomassas alocadas para reprodução sexuada e assexuada, bem como a influência da clonalidade em outros aspectos da história de vida de uma planta tem trabalhado com resultados em número ou investimento em biomassa dos propágulos (brotos ou estruturas sexuadas de reprodução) (COELHO et al., 2000; THOMPSON; ECKERT, 2004; COELHO et al., 2005; CAO; WORLEY, 2013). Entretanto, muitos aspectos prévios à formação dos propágulos podem ser afetados pelos *trade-offs* e não estarem refletidos de maneira adequada nos investimentos finais.

Esse pode ser o caso, por exemplo, dos traços florais, diretamente ligados à reprodução sexuada. Apesar do grande reconhecimento da variabilidade intraespecífica em traços funcionais das plantas (KUPPLER et al., 2016) a literatura reforça uma menor variação com relação à plasticidade dos traços florais (GIVINISH, 2002; ARMBRUSTER et al., 2004). Essa menor variabilidade deve-se, principalmente à forte seleção estabilizadora (ANDERSON, 1994; CRESWELL, 1998) ligada à garantia do reconhecimento pelos polinizadores e do sucesso da reprodução sexuada (CHARLESWORTH; CHARLESWORTH, 1987).

Entretanto, outros estudos mostram que a variação ambiental é uma importante fonte de variação para os traços florais. Essa variação pode advir de possibilidade e quantidade de interações bióticas (THOMPSON, 2001; COELHO; MALVES, 2015; KUPPLER et al., 2016). Entretanto, também podem ser geradas por diferentes condições de recursos (FUJITAKA ; SAKAI, 2007; BROOKES et al., 2010; XIE et al., 2016).

Dessa forma, dado que a manutenção da reprodução sexuada em plantas clonais e os antagonismos entre as reproduções sexuada e assexuada ainda não estão claramente resolvidos (ERIKSSON, 1997; CHARPENTIER, 2002; SILVERTOWN, 2008; VALLEJO-MARÍN et al., 2010; BARRET, 2015), a relação da clonalidade e da variação dos traços florais e de outros traços de história de vida das plantas ainda é um estimulante foco de pesquisa e pode gerar grande quantidade de informação acerca da evolução dos modos reprodutivos e de sua manutenção ao longo do tempo evolutivo.

Neste sentido, esta tese de doutorado busca responder como a integração clonal está relacionada com a variação nos traços florais e em outros traços de história de vida de uma planta altamente distribuída no globo, *Eicchornia crassipes*, e como essa relação pode explicar o grande sucesso reprodutivo e de colonização de ambientes dessa espécie. Para tanto, as informações aqui contidas estão divididas em três capítulos. Os dados coletados para a construção de todos os três capítulos foram obtidos por meio de experimentos em casa de vegetação, que nos auxiliaram a controlar os fatores externos, que não eram de nosso interesse. No primeiro capítulo, foram avaliadas as respostas dos traços florais à integração clonal, comparando suas medidas entre rametes ligados às plantas-mãe a outros que tiveram suas conexões experimentalmente rompidas. No segundo capítulo, avaliou-se se a variação intra-individual dos traços florais está ligada à integração clonal e à capacidade que este traço confere de existência de translocação de recursos entre plantas-mãe e rametes filho. Por fim, no terceiro capítulo, avaliou-se como a integração clonal interfere na alocação de biomassa para as diversas partes do corpo da planta, e como essas relações estão ligadas ao sucesso de indivíduos de *E. crassipes.*

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SEGUNDA PARTE - ARTIGOS

ARTIGO 1

WHAT ARE THE CONSEQUENCES OF CLONAL INTEGRATION FOR FLORAL TRAITS AND REPRODUCTIVE INVESTMENT OF A BROADLY DISTRIBUTED AQUATIC PLANT?

WHAT ARE THE CONSEQUENCES OF CLONAL INTEGRATION FOR FLORAL TRAITS AND REPRODUCTIVE INVESTMENT OF A BROADLY DISTRIBUTED AQUATIC PLANT?

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ABSTRACT

The ability of offspring generation by sexual and asexual reproductive modes widens up plants reproductive potential, since it makes feasible resource allocation in distinct reproductive modes under different environmental conditions. Clonal reproduction, however, has been considered a sink of resources that decreases sexual reproduction due to potential trade-offs between reproductive modes. On the other hand, clonality may allow clonal plants to share resources such as water, photosynthates and nutrients among individual subunits, generating variability on key traits, as floral shape and display. The objective of this work was to examine wether clonal integration affects floral traits on a widely distributed aquatic plant, and how this influence may affect plants success. We found that clonal ramets exhibited larger and more robust flowers than isolated ones, but the number of flowers per inflorescence did not change in comparison to isolated plants. These changes are discussed as mechanisms of reproduction enhancement. We conclude that clonal integration changes *Eicchornia* *crassipes* resources investment on sexual reproduction and may be identified as a persistence strategy for aquatic plants

Keywords: allocation theory; life-history theory; plant reproduction; reproductive modes;

1. INTRODUCTION

Many perennial flowering plants reproduce sexually and asexually (e.g. by clonal growth) (Richards, 1986), and the balance between clonal and sexual reproduction can vary amongst populations of a species (Eckert, 2002). Clonal plants are well recognized for their ability of producing offspring via sexual and asexual processes (Harper, 1977). This feature widens plants reproductive potential, as it makes feasible resource allocation in distinct reproductive modes (sexual or asexual) under different biotic and/or abiotic conditions (Abrahamson, 1980; Coelho et al., 2005; Ikegami et al., 2008). Thus, clonality may be considered as a key life history trait, since it can allow plants to reproduce and sustain propagules, even under harsh environmental conditions (Coelho et al., 2008a,b). For example, aquatic habitats often experience significant fluctuations such as seasonal or aseasonal drying and/or flooding of habitats (Coelho et al., 2005), and many aquatic plant species exhibit adaptations that facilitate survival and reproduction. When these alterations consist of variation in water level and rapid changes in the speed of water currents during floods, genets of clonal plants may only survive these forms of disturbance through clonal fragmentation and the dispersal of vegetative propagules (Eckert et al., 2016).

As a reproductive process, clonal reproduction has been discussed as a sink of resources that decreases sexual reproduction potential as the allocation of nutrients in the production of asexual propagules shortens their availability for flower and fruit production (Bai et al., 2009; Liu et al., 2009). Seminal papers have found that clonality may allow plants to share resources such as water, photosynthates and nutrients among individual subunits, called ramets (Alpert, 1996, 1999; Yu et al., 2002; Coelho et al., 2007, 2008). This would even lead single genets to exhibit 'labour division' among their ramets (Stuefer, 1998), and could, in fact, improve sexual reproductive investment (Demetrio et al., 2014).

In adittion, floral traits are usually seen as exhibiting the lowest variation pools in plants, usually kept under stabilizing selection (Anderson, 1994; Creswell, 1998), since a more constant flower morphology allows pollinator recognition and posterior exchange of pollen grains between flowers (Armbuster et al., 2004), improving sexual reproductive success. On the other hand, variability in floral traits may generate a larger pool of options for natural selection, as it may allow different plant-animal interactions frequencies among plant individuals (Gómez and Perfecti, 2012). This may turn more trait-diverse populations prone to endure environmental changes by the ability of attraction of different groups of pollinators (Hooper et al., 2005; Kuppler et al., 2016).

Many of the plants life history traits exhibit strong relationships with environmental conditions. In *Eicchornia crassipes* (Pontederiaceae), for example, plants morphology is driven by plant density (Alvarenga et al., 2013), and sexual and asexual reproduction investment can be influenced by leaf damage and pollination (Buchanan, 2015). Furthermore, floral traits as flower size, and flower biomass (Malves and Coelho 2015; Xie et al., 2016) and flower display (accounting for characteristics like flower colour and scent) may also be affected by environmental conditions. The influences of asexual reproduction on sexual reproduction has already been demonstrated on the ecological literature (e.g., Reekie and Bazzaz, 1987; Bazzaz, 1997; van Kleunen et al., 2002). However, these studies commonly deal with the relationship between the number of sexual and asexual propagules, not examining the influence of asexual propagation on sexual reproductive structures traits, such as flower morphology, biomass, and size.

In this context, the objective of this paper was to assess if clonal integration by structures like stolons affects the floral traits and reproductive investment of a widely distributed aquatic plant. Based on the premises that (i) higher resource levels would cause significant increases on sexual reproduction (Thompson and Eckert, 2004), and (ii) variation in resource uptake among individuals can cause positive covariation between competing functions (asexual and sexual reproduction) (Reznick et al., 2000), we hypothesized that: i) ramets attached to their mother plants would produce more, higher and more robust flowers, as clonal integration would generate translocation of assimilates in a basipetal movement, enhancing ramet sexual reproduction; ii) isolated ramets would invest in asexual reproduction, as a higher number of ramets in a clone will act as a bigger net of nutrients assimilation.

2. MATERIALS AND METHODS

2.1 Study species

Eichhornia crassipes (Mart.) Solms (Pontederiaceae), popularly known as Water-hyacinth, is a floating clonal aquatic plant (Pott and Pott, 2000) native to the basin of Amazon river (Barret, 1980). It is considered as an invasive plant because of its great reproductive and colonization potential, with seeds that lasts near 15 years viable in the soil (Pott and Pott, 2000).

Eicchornia crassipes flowers exhibit a peculiar flower morphology condition called tristyly, which generates three different flower morphs (Barret, 1980). These different forms are recognized by peculiar relationships between stylus and stamen lengths. In our study site, the sampled population only exhibited mid-style morphs, what means that the stylus length is intermediate in relation to short and long stamens length (Fig. 1). *Eicchornia crassipes* flowers also exhivit a differentiated petal, hereafter called banner petal, possessing a conspicuous yellow spot that is considered as a nectar guideline (Barret, 1980).

2.2 Terminology

Clonal plants are able to produce offspring by sexual and asexual modes. A genet is an individual formed through sexual reproduction by seed formation. The ramet, it should be noted, is defined as an individual formed by vegetative growth that can survive as a physiologically independent unit in relation to its parental plant (sensu Harper, 1977). We considered the rosette (shoot forming), its associated root system, and its associated caulinar system (composed by a stolon fragment), as a ramet. Hereafter we call all the ramets of rosettes.

2.3 Ramets sampling

The ramets used for greenhouse experiments were sampled at a population of *E. crassipes* located at Represa do Funil, Ijaci, Minas Gerais. We selected adult ramets (identified by the presence of newly produced sexual reproductive structures or their remainings - as old floral scapes) with no signs of foliar herbivory or diseases. We selected a total of 90 ramets that were put in plastic bags filled with some water to avoid root dessication and took them to a greenhouse at Federal University of Lavras in order to carry the experiment.

2.4 Greenhouse experiment

We distributed the ramets among 18 pots filled with 17 L of tap water. Ramets were left inside the greenhouse for a period of two weeks without any interference in order to allow an acclimation period. After this time period we selected 18 ramets of similar size that were isolated in pots filled with 17 L of tap water. The ramets were cultivated until the production of asexual offspring. This first generation of ramets produced under greenhouse conditions are here referred as “mother ramets” from hereafter. Mother ramets remained attached to their parental ramets until they also produced a generation of asexual offspring.

After this, mother ramets and their offspring were split off from parental ramets. This was the basic unit of our experiment, a group of a mother ramet attached to it first asexual offspring. These groups were also distributed among 18 pots filled with 17 L of tap water. We set two treatments on these mother-daughter ramets groups. The first one, ‘isolation treatment’, consisted on nine daughter ramets that were experimentally split off from their mother ramets. The second one, ‘clonal treatment’, consisted on the maintenance of nine groups as originally conceived, with the daughter ramet attached to the mother ramet.

Plants were monitored during 12 weeks. Every group was checked and buds and flowers number were recorded. We also collected buds and flowers and dried them on oven at 60ºC in order to obtain the biomass of reproductive structures. After counting every structure was removed of every ramet under clonal and isolated condition in order to maintain the experiment original conditions.

2.5 Flower traits measurement

After sampling, the flowers were promptly taken to the laboratory, where floral length, banner petal length, nectar guideline length, short and long stamens length, and stylus length were measured with a digital caliper with 0.001 cm precision.

2.6 Data analysis

All variables were tested for normality with Shapiro-Wilk tests. In order to evaluate the influence of clonality on *E. crassipes* reproductive processes we applied GLMM’s with Poisson distribution for flowers and buds number and with Gaussian distribution for floral traits and for flowers and buds biomass. For every model, the ramet was inserted in the model as a random factor, while the treatment (isolated or clonal condition) was inserted as a fixed variable. All analyses were carried on R Statistical Software (R Core Team, 2016), using the package lme4.

3. RESULTS

Investment in reproductive structures and most part of floral traits showed clear and significant responses to clonal integration interruption through stolons severing. Individuals that remained attached to mother ramets presented a bigger floral display and bigger floral traits in comparison to individuals that had their stolons cut. Sexual investiment was also higher, with ramets connected to mother ramets bearing flowers that were more robust than those generated by ramets without connection to mother ramets.

The number of reproductive structures was not different for clonal and isolated plants. Both bud (χ^2 = 1.30, p = 0.25), and flower numbers (χ^2 = 2.08, p = 0.14) did not differ between clonality treatments. Reproductive structures biomass, however, showed a different pattern. Bud biomass did not differ between treatments (χ^2 = 3.33, p = 0.061), but flower biomass was significantly higher for individuals that remained attached to mother ramets in comparison to those which stolon connections were cut out (χ^2 = 44.05, $p < 0.001$, Fig 2).

Ramets isolation from mother ramets presented a strong influence on floral traits. Flower length was smaller in ramets without connection (χ^2 = 55.67, $p < 0.0001$, Fig. 3A), as flowers estimated to be 1.16 cm shorter than flowers in clonal condition. The banner petal was also smaller on individuals that were kept under isolated condition (χ^2 = 16.85, $p < 0.0001$, Fig. 3B). This was expected, as banner petal lenght was positively correlated with flower size (r = 0.72), which was bigger on clonal plants. Nectar guides on banner petals were also smaller on flowers on isolated individuals (χ^2 = 3.91, p = 0.047, Fig. 3C). Nor long (χ^2 = 3.49, p = 0.061), or short (χ^2 = 0.49, p = 0.48) stamens showed differences in lenght between flowers generated by clonal and isolated plants. However, styles length was larger in flowers arising from clonal individuals (χ^2 = 7.69, p = 0.0005, Fig. 3D).

4. DISCUSSION

Clonal growth has been largely discussed as a process that can potentially undermine sexual reproduction on plants, our results, however, show that clonal integration may be an important mechanism improving flowers production and enhancing floral display. The antagonisms between sexual and asexual reproduction are not simple and a clear resolution is not available yet (Eriksson, 1997; Silvertown, 2008; Vallejo-Marín, 2010). Many mechanisms have been suggested in order to explain these relationships. From the point of view of the developmental state (Watson, 1984), meristem limitation may be responsible for apparent trade-offs between sexual and asexual reproduction, as plants engaged in asexual reproduction would not invest in flowering, as a meristem commited to a new shoot growth and maintaining would not be available for flower shoots development. This case has been specifically analysed for *E. crassipes* by Watson (1984, 1988). She argued that a developmental program was the main driver of the allocation patterns for new ramets or flowering shoots in *E. crassipes*, and that carbon uptake would not mean a true limitation for plants life history processes. This would generate an absence of influence of sexual reproduction on vegetative growth, because carbon would be enough for investment in both functions. This seems to hold true for our data when the number of flower and vegetative buds are analysed, as both isolated ramets and those ones that remained attached to mother ramets produced a similar number of vegetative offspring, and also a similar number of flowers per ramet.

However, our study has shown that flower biomass and length were higher on ramets attached to the mother plants than the isolated ones. This leads us to point of view of the theory resource allocation (Gadgil and Solbrig, 1972; Stearns, 1992; Weiner, 2004), which has traditionally been considered to be a ratio driven process: ‘partitioning’. According to this perspective, a plant with a given amount of resources at any point in time partitions these resources among different structures or activities (Weiner, 2004, 2009).

In this sense, the lack of resources would obligate the ramet to invest in sexual or asexual reproduction, as under a limitation on resources for metabolism and biomass gain the increase in resource allocation to one function would reduce that to another functions (Bai et al., 2009; Liu et al., 2009). Clonal integration may decrease this resource competition among ramets on a site, and growth of ramets, for example, will be controlled by the internal allocation of resources within the integrated plants as a whole. This may allow ramets attached to their mother ramets to produce larger and more robust flowers, even when sharing resources to the mother ramet, because they will both act as a single productive unit, possessing a bigger root system, that would be more efficient on nutrient uptake from water, in contrast to an isolated ramet. In the specific case of *E. crassipes*, flowering processes are linked to a low nutrient content on water (Richards, 1982; Watson and Brochier, 1988), and a bigger root system would allow ramets within a clone to maximize their nutrients uptake. This pattern is strong for other aquatic plants, as Xiao et al. (2011) showed that for *Spartina alterniflora* ramets, integration was responsible for an increase on flower production when ramets were linked to mother ramets. Life history theory has emphasized the importance of allocation patterns to plant reproductive processes (Abrahamson and Gadgil, 1973) and size-dependent sex allocation is widely observed among flowering plants (Klinkhamer and de Jong, 1997). Allocation is the central concept in life history theory (Stearns, 1992) and large organisms tend to make more of everything than those that are small (Reznick et al., 2000). Allocation patterns reflect ecological strategies that arisen as a result of different selection pressures and constraints (Bonser and Aarssen, 2001). The primary effects of environment on plants reproductive processes occur via plant size (Weiner et al., 2008), since plant size can affect the probability of a plant flowering onset (Weiner et al., 2009), and determines a plant reproductive output when it does occur (Weiner, 1988). In our experiment, plants that remained attached to mother ramets were bigger than isolated ramets (G. R. Demetrio and F. F. Coelho, pers. obs.), and this may have enhanced sexual investment through the movement of resources from mother to daughter ramets, and the investment of resources stored on the stolons. This would be due to the ability of clonal organs to enhance resource uptake and storage (Grace, 1993).

In relation to flower traits, clonal strategy has been argued as possessing a potential impact on mating processes and patterns, what would affect reproductive success (Handel, 1985; Mori et al., 2009). A higher number of flowers per ramet would increase the rates of geitonogamy, since pollinators tend to visit a smaller proportion of flowers in patches with larger floral displays (Ohashi and Yahara, 1998; Karron and Mitchel, 2012). However, rates of pollinators visitation may vary in relation to features of floral design, as floral size. Different insect taxa show very different responses to variation in floral size and some groups show a marked preference for larger floral sizes (Thompson, 2001). In our experiments stamens length was not different among isolated and ramets linked to mother ramets, but style length was bigger in ramets that remained attached to mother plants. This may be expected as style length and position are plastic in several taxa (Barret et al., 2000).

In fact, floral traits are selected to ensure sexual reproduction, because cross-fertilization increases genetic variability, which is advantageous to offspring (Charlesworth and Charlesworth, 1987). In our study, floral traits involved with pollinators attraction, as flower size (Rosas-Guerrero et al., 2011), and nectars guidelines lenght were bigger on ramets that remained attached to mother plants, what may suggest that clonal integration may be important on enhancing pollinators visitation, and so, sexual reproduction, as a higher floral display may allow higher pollination rates (Klinkhhamer et al., 1989). This may be highly important, because clonal growth itself influences ramets shape and distribution, what may affect crossing opportunities between individuals. To understand how the variation in floral traits affects the variability and success of flower visitors interaction patterns in *E. crassipes* is a task for future studies.

We conclude that clonal integration changes *E. crassipes* abilities of uptaking and investing resources on sexual reproduction and is a key life history trait, as it allows plants to ensure greater sexual investment. This process may be identified as a persistence strategy for aquatic plants (Coelho et al., 2005). Furthermore, clonal integration also generates variability in floral traits, what may affect plant's reproductive success by mediating different plant-animal interactions amongst plant individuals (Gómez and Perfecti, 2012).

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FIGURES AND LEGENDS

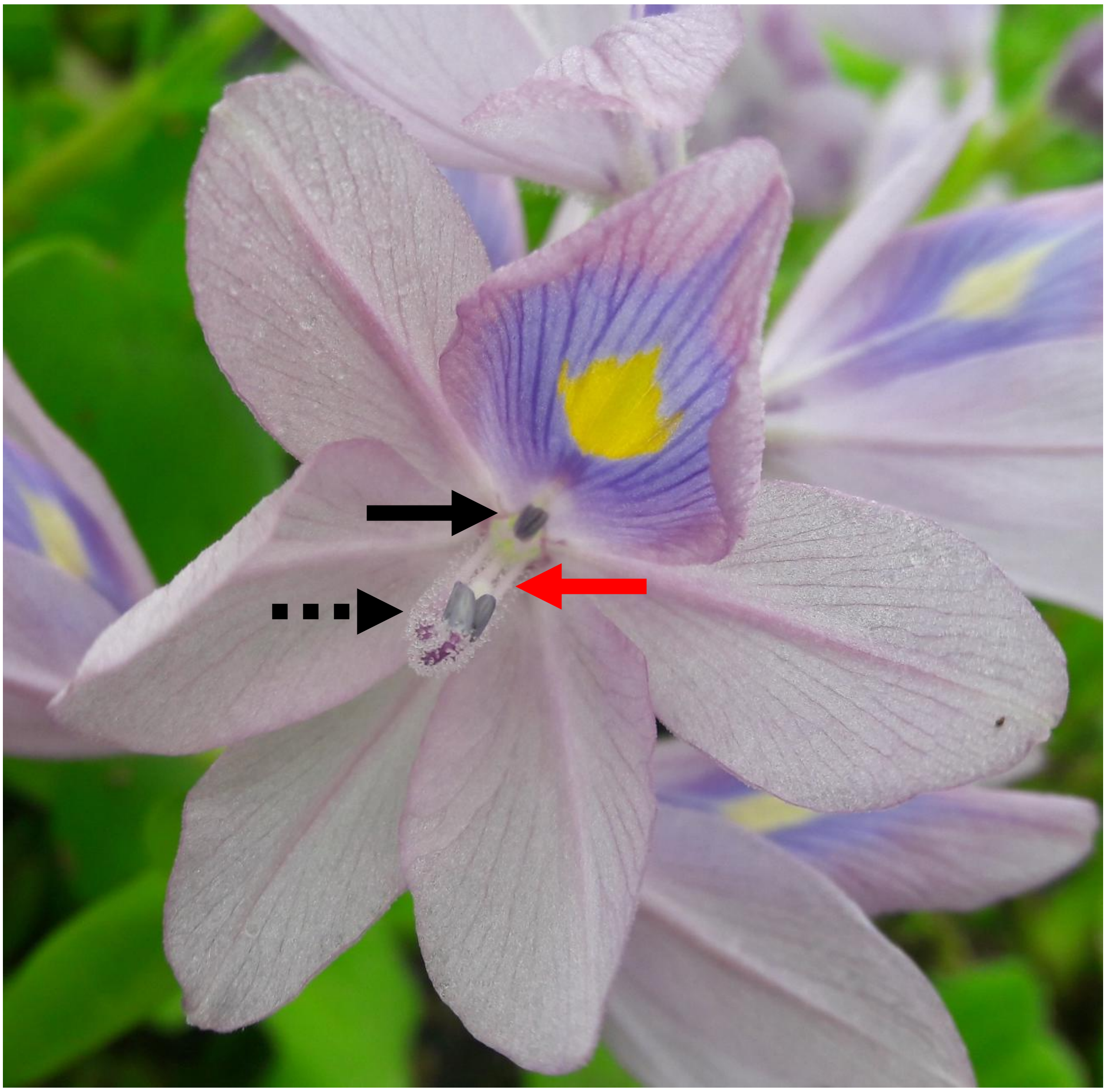

Figure 1. General flower morphology of *Eicchornia crassipes*. Stamens are pointed with black arrows. The solid line shows the short stamens, while the dashed line points the long ones. The red arrow points the style head, just below the long stamens whorl.

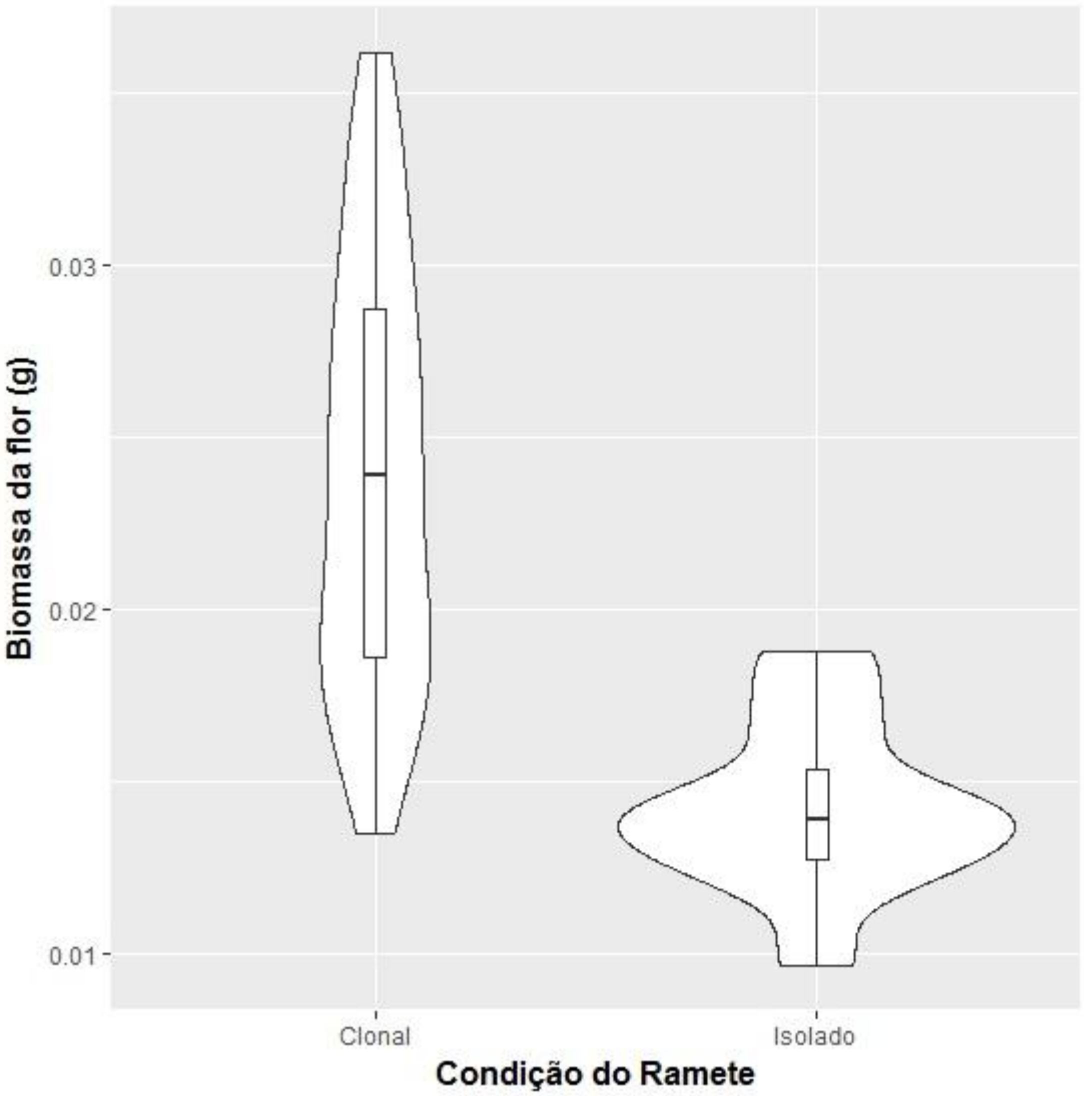

Figure 2. Flowers biomass (g) in ramets that remained attached to mother plants (C) *versus* those ones which stolons were severed (I).

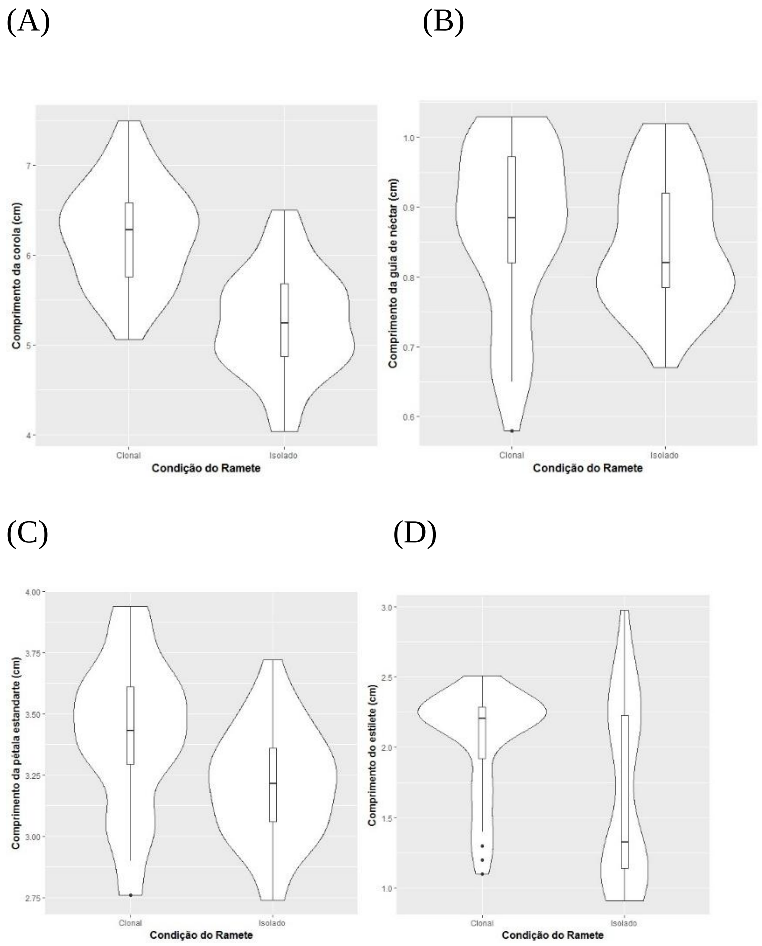

Figure 3. Floral traits comparison between ramets that remained attached to mother plants (Clonal) and those which stolons where severed (Isolated): (A) Flower length (cm); (B) Banner petal length (cm); (C) Nectar guideline length (cm); (D) Styles length (cm). The bomxes represent the quartiles, the line on the box represent the median, vertical lines represent the mean error, dots represent outliers and the area around the boxes represent the data dispersion.

ARTIGO 2

INTRA INDIVIDUAL DIFFERENCES IN FLOWER TRAITS ON *Eicchornia crassipes* (Pontederiaceae) ARE EXPLAINED BY CLONAL INTEGRATION, RESOURCE AVAILABILITY AND FLOWER POSITION

INTRA INDIVIDUAL DIFFERENCES IN FLOWER TRAITS ON *Eicchornia crassipes* (Pontederiaceae) ARE EXPLAINED BY CLONAL INTEGRATION, RESOURCE AVAILABILITY AND FLOWER POSITION

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ABSTRACT

Intra individual variation on floral traits are strongly linked to plants fitness, and thus, play a central role on sexual selection. This variation may arise from a variety of sources, as architectural constraints, such as flower position on the inflorescence axis, and environmental conditions. In relation to the environment influences on floral traits, the most common causes of variation are linked to the presence of pollinators, to foraging patterns and to the availability of local resource pools. The aim of this work was to evaluate how clonal integration and resource impoverishment via defoliation influences floral trait variation on individuals of *Eicchornia crassipes.* We set greenhouses experiments and acessed data about flower display and primary sexual structures production. Data was analysed via general linear mixed modelling and model selection. We found that architecture and resource availability are important to shape floral traits in *E. crassipes* and that clonal integration may be a stability source for these characters. We conclude that sexual reproduction is strongly influenced by clonal integration and resource availability in *E. crassipes* and suggest that southern Brazilian populations may be not limited by pollinators absence.

Keywords: clonality; flower morphology; intraindividual variation; sexual reproduction;

1. INTRODUCTION

Ecological and evolutionary studies have long emphasized variability among populations and/or specific functional traits (Newton et al., 1999; Dai et al., 2016; Delgado-Dávila et al., 2016). However, between individual differences has gained attention and recently incorporated to ecological studies (Violle et al., 2012; Kuppler et al., 2016). This variation is of high importance for evolutionary processes, mainly because natural selection will favour the allocation of finite resources to different functions in a way that maximizes plant fitness (Stearns, 1992; Roff, 2002). In this sense, plant traits as flowers size and patterns of sexual investment within the individual deeply influence plant mating, and are, thus, subjected to natural and sexual selection (Thomson, 1988; Ohara and Higash, 1994; Torices and Méndes, 2011).

Within-plant variation can affect interaction patterns (Biernaskie et al., 2002), and great part of this variation seems to be absorbed by flowers and inflorescences (Winn, 1991; Diggle, 1995, 1997), when compared to inter individual levels. It is comprehensive to understand this high level of variation when one recognizes that the flowers are not a unique organ, but a group of several independent organs covarying (Berg, 1956, 1960; Ishii and Morinaga, 2005) exhibiting high degrees of integration in working together as reproductive structures.

This variation of floral traits within individuals/inflorescences can reflect differences in environmental and/or ecological factors, such as plant size (Zhao et al., 2007; Zhang et al., 2014), pollinator behaviour, out-crossing rates (Iwaizumi and Sakai, 2004) and local resource environments (Austen et al., 2015). These differences can also be explained by architectural effects of resource conditions among flowers at different positions (Brunet and Charlesworth, 1995; Mazer and Dawson, 2001). The internal resource gradients caused by proximal-to-distal decline in vasculature size (Byrne and Mazer, 1990; Wolfe, 1992), specially on plants that show acropetal flowering (Thomson, 1989; Wolfe, 1992; Zhao et al., 2008) as *Eicchornia crassipes*, may result in a resource gradient in which basal flowers are prote to receive more resources than those located on more distal positions, acting like a resource sink.

In this sense, plants in different resource conditions are prone to show different patterns of intraindividual variation. In clonal plants, clonal integration may represent an important mechanism of resource exploitation allowing the exchange of nutrients and photosynthates among parental and daughter ramets, including the differentiation in function for each ramet composing a clone, a process called labour division (Stuefer et al.,1994; Stuefer, 2002; Demetrio et al., 2014), what may influence the intra-individual floral variation patterns.

The objective of this work was to examine how clonal integration, resource availability and flower position interact to determine intra-individual variation of sexual reproductive traits. We hypothesized that i) ramets that remain attached to parental plants should show lower floral variation due to high availability of resources when compared to defoliated and isolated ramets and, ii) defoliated ramets should present the highest floral position influence on sexual reproductive traits because flowers located in more proximal positions on the inflorescence would represent a strong resource drain (Buide et al., 2008; Brookes, 2010; Cao et al., 2011).

2. MATERIALS AND METHODS

2.1 Study species

Eicchornia crassipes (Mart.) Sölms. is a free-floating aquatic macrophyte (Barret, 1980a,b, Pott and Pott, 2000), native from the Amazon river basin (Pott and Pott, 2000). In the last years, it has been recognized as an aggressive invasive species (Holm, 1977), reaching a worldwide distribution (Barret, 1989). This increase in its geographic range may be related to the vigorous reproductive processes, that occur both via asexual and sexual pathways (Barret, 1980a; Watson, 1984). Sexual reproduction is not constrained by oligotrophic habitats (Watson and Brochier, 1988), and does not look like to be a weakening factor for asexual reproduction (Watson, 1984). Sexual reproductive structures appear grouped in an inflorescence that generally arises from the apical meristem and bears showy light purple flowers that open in an acropetal order (from the bottom to the top of the inflorescence).

2.2 Plant sampling

All plant material was collected from a large monospecific mat located at Represa do Funil, Ijaci, Lavras, Minas Gerais, Brasil. Plants were collected and previously washed in the field. We selected adult ramets (identified by the presence of newly produced sexual reproductive structures or their remaining - as old floral scapes) with no signs of foliar herbivory or diseases. We selected a total of 90 ramets that were put in plastic bags filled with some water to avoid root desiccation and took them to a greenhouse at Federal University of Lavras in order to carry the experiment.

2.3 Greenhouse experiment

We distributed the ramets among 18 pots filled with 17 L of tap water. Ramets were left inside the greenhouse for a period of two weeks without any interference in order to allow an acclimation period. After this time, we selected 18 ramets of similar size that were isolated in pots filled with 17 L of tap water. The ramets were cultivated until the production of asexual offspring for each of the selected ones. This first generation of ramets produced under greenhouse conditions are referred as “parental” from hereafter. Parental ramets remained attached to the ramets from which they derived until they also produced a generation of asexual offspring.

After this, parental ramets and their offspring were isolated, forming the basic sampling unit of the experiment. These groups were placed in 27 pots filled with 17 L of tap water. After this, we set three treatments on these parental-daughter ramets groups. The first one, ‘isolation treatment’, consisted on nine daughter ramets that were experimentally split off from their parental ramets. The second one, ‘clonal treatment’, consisted on the maintenance of nine groups as originally conceived, with the daughter ramet attached to the mother ramet. The third one, ‘Defoliation and Isolation treatment’, consisted on nine daughter ramets that were experimentally splitt off from their parental ramets and also had all of their leaves removed at the beginning of the experiment, simulating a resource shortage. The experiment was carried out during three months and plants were daily checked in order to sample for flowers, as flowers last for only 24 hours.

2.4 Flower traits measurement

Every time a plant flowered, flowers were promptly taken to the laboratory. We assigned a number for each flower in an inflorescence, in a scale from one to seven (the maximum number of flowers we found in a unique inflorescence), in what one represented the most bottom flower and the subsequent numbers represented the upper positions in the inflorescence. In the laboratory, each flower was dried to constant weight and biomass was measured in a precision balance. We also measured each flower’s corolla length, banner petal length, nectar guideline length, short and long stamens length, and stylus length with a digital caliper with 0.001 cm precision for all flowers in each treatment.

2.5 Data analysis

All variables were tested for normality with Shapiro-Wilk tests. In order to evaluate the influence of flower position on *E. crassipes* floral traits and its possible interactions with clonal integration, we applied GLMM's with Gaussian distribution for all variables. In order to validate our data distribution choices we carried out normality tests for the residuals, in which we verified normal distributions for all of them. For every model, the ramet was inserted in the model as a random factor, whereas flower position, treatment (isolated, clonal condition, or defoliation), and their interaction term were inserted as a fixed variable. After that, we ran a model selection, using the function dredge, of the package MuMin, for each response variable, and the best models were considered to be those composing a group containing the model with the lowest AICc value and those whose delta were lower than 2. The variables were discussed according to its appearance in the candidate models and their effect direction was discussed based on the summary of the model with the lower AICc among the candidate models, if there were more than one. All analyses were carried on R Statistical Software (R Core Team, 2016), using the package lme4.

3. RESULTS

Flower position appeared in the candidate models as a potential explanation for intra-individual floral traits variation for all measured traits in this experiment. However, trait responses did not show a consistent pattern. Clonal integration also had effects on floral traits, and the interaction of flower position and clonal integration were only statistically significant for secondary sexual reproductive traits (floral display and pollinator attractiveness) (Table 1).

In regard to sexual primary function, here listed as long stamens whorl length, short stamens whorl length, and style length, flower position and treatment terms of the models showed influences, with the interaction being not significant (Table 1). For these three traits, the candidate models showed that flower position had a negative effect, indicating that flowers tend to produce shorter structures on apical flowers when compared to the basal ones. The best model, however, only included the treatment, showing that the resource depletion has a strong influence on the structures size in comparison to flower position influence (Table 2). Here, an interesting pattern arose. ‘Defoliation’ treatment promoted an increase on the size of the structures when compared to ‘Clonal’ treatment, while the ‘Isolation’ treatment, in general, showed shorter structures than those of the flowers generated by plants of the ‘Clonal’ treatment (Table 2).

In relation to sexual secondary function, related to floral display and pollinators attraction, here listed as floral biomass, corolla length, banner petal length and nectar guideline length, the interaction term was significant in all of the best models (Table 1). All the measured floral traits, with exception of floral biomass, decreased in function of floral position from the bottom to the top of the inflorescence, and this decrease was stronger on plants of the ‘Defoliation’ treatment (Table 2). Nectar guideline length was the only floral trait of ‘Defoliation’ treatment plants that had a smoother decrease in relation to the treatments, with ‘Isolated’ treatment plants showing the strongest reduction in nectar guidelines length with the increase of flower position (Table 2).

4. DISCUSSION

Our results showed clear patterns of intra individual variation on floral traits, affected by treatments. When the traits are related to the flower primary function, the sexual reproduction *per se*, flower position had no influence, while resource availability was a driver for stamens and style sizes. In the case of traits of floral display, associated to pollinator attraction, a combination of flower position and resource availability was the main driver for traits size variation.

Floating plants are constantly submitted to a variety of habitats and may anchor on patches with low resource availability. Life-history theory would predict strong trade-off patterns (Stearns, 1992; Roff, 2002), with some plant activities being impaired by allocation shortage. However, even after a plant made its “decision” on how much to allocate to each life history main process (survival, growth, and reproduction), there are many “sub-functions” that are also prone to suffer trade-offs. In sexual reproduction, for example, the individual has to divide the allocated resources among, e.g., pollinators attractiveness, floral stalks and floral reproductive organs (anthers and ovaries). This would generate a hierarchical resource allocation (Cao and Worley, 2013).

In our experiment, the plants under the lowest resource availability (those that were splitted of their parental ramets, precluding resources sharing via clonal integration, and had their leaves removed), exhibited little influences of intra individual investment on sexual reproductive organs size, what did not happen to floral display traits. This could represent an evolutionary response to environmental stress. Some disturbs may promote sexual reproduction on aquatic plants (Eckert et al., 2016), and under stress conditions, aquatic plants usually allocate a great amount of resources to sexual reproduction, when compared to asexual reproduction (Coelho et al., 2005).

In some cases, severed ramets can produce more inflorescences under nutrient and water limitation, as Evans (1992) reports for *Hydrocotile bonariensis*. In this sense, *E. crassipes* ramets may have perceived defoliation and absence of clonal integration as a high stress level, what may have biased the decision towards investment on sexual reproductive organs. In this sense, why the ramets did not invested on floral display, the main dispositive for pollinators attraction? Besides the apparent importance of pollinator foraging to extensive clonal heterostylous species (Barret, 2015), a previous study carried out in an extensive range of *E. crassipes* found a low number of populations showing insect visitation (Barret, 1980b), and clones of *Eicchornia crassipes* ramets are reported in literature as possessing weak or absent self-incompatibility (Barret, 1980a), what promotes the production of seeds even under pollinator absence.

These selfing behaviour is strong on southern Brazilian populations, where self and crossed pollinated plants do not appear to show fertility differences (Barret 1980a). A high occurrence of selfing was also registered for a population of *E. crassipes* that occur on a seasonal marsh, where seeds, resistent propagules, are formed right before the dry season (Barret 2015). Cao et al. (2011) also showed an increase on sexual reproductive structures (ovule and pollen grains) within defoliated ramets. In this way, for the plants in our experiment, it seems to be more important to guarantee the production of functional reproductive organs than to attract pollinators, since seeds will be set in its absence.

'Isolated' ramets produced smaller structures when compared to 'Clonal' and 'Defoliated' ramets, and an effect of flower position on decreasing floral traits from the bottom to the top of the inflorescence. Clonal integration is central for clonal plants functioning (van Groenendael and de Kroon, 1990; Liu et al., 2016) as they enable physiological integration via the maintenance of connections (Dong, 2011). These complex patterns of translocation (Dong, 1996, 2011), may promote an increase in resource foraging (Liu et al., 2016), and even a labour division (Stuefer et al., 1994; Demetrio et al., 2014; Liu et al., 2016). In the case of the 'Isolated' plants, a ramet bearing a limited number of roots and leaves was entirely responsible for nutrient uptake, photosynthesis and the allocation of resources to sexual and asexual reproduction. In the absence of the connection with parental plants, the amount of resources available for these functions in each daughter ramet decreased, generating the pattern of flower shortening that was observed along the inflorescence axis, that occurred in a softer way in 'Clonal' ramets.

We conclude that floral traits show great intra individual variation in *E. crassipes* ramets, and that this variation is driven by architecture, mediated by flower position, resource availability, and by the nature of the trait function. Traits that are linked to the ultimate output of a function, as stamens and style in relation to sexual reproduction in high fertile self-pollinated plants, suffer less variation than those that are linked to secondary functions, as pollinator attractiveness, in this case. Our data corroborate the idea that sexual reproduction is strongly influenced by environmental conditions in *E. crassipes*, (Barret, 1980a), and suggest that populations of water-lily may be not limited by pollinators absence.

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TABLES

Table 1. Candidate models for each relationship among *Eicchornia crassipes* floral traits and explanatory variables (flower position, treatment and interaction term) showing the variables present in each model.

Trait	Model	Intercept	Flower Position	Treatment	Flower Position*Treatment	AICc	Delta	Weight
Floral biomass	1	0.01758	-0.0002673	+	+	-1118.9	0	0.377
	2	0.01745	-0.0002283	+		-1118.6	0.24	0.334
	3	0.01670		+		-1118.3	0.54	0.228
Flower length	1	5.554	0.09032	+	+	304.2	0	0.818
Banner petal length	1	3.229	0.01478	+	+	194.6	0	0.996
Nectar guide length	1	0.8407	-0.007613	+	+	62.8	0	0.994
Long stamens length	1	2.216	-0.02395	+		-26.4	0	0.688
Short stamens length	1	0.9371		+		65.3	0	0.56
	2	0.9975	-0.01874	+		66.1	0.76	0.381
Style length	1	1.794		+		160.2	0	0.54
	2	1.716	0.02441	+		161.3	1.09	0.313

Table 2. Summary coefficients of each of the best models suggested by the model selection regarding the relationships among *Eicchornia crassipes* floral traits and the explanatory variables.

Trait	Component	Estimate	*t* value	*P*
Floral Biomass	Intercept	0.0175	20.95	< **0.001**
	Treatment (Defoliated)	0.0084	6.947	< **0.001**
	Treatment (Isolated)	-0.011	-10.28	< **0.001**
	Flower Position	-0.0002	-1.252	0.213
	Treatment (Defoliated) * Flower Position	-0.0002	-0.792	0.43
	Treatment (Isolated)* Flower Position	0.0005	1.553	0.123
Corolla lenght	Intercept	5.554	20.55	< **0.001**
	Treatment (Defoliated)	0.512	1.450	0.14966
	Treatment (Isolated)	0.0725	0.217	0.82883
	Flower Position	0.0903	1.463	0.14613
	Treatment (Defoliated) * Flower Position	-0.261	-2.922	**0.00416**
	Treatment (Isolated) * Flower Position	-0.223	-2.159	**0.03289**
Banner Petal lenght	Intercept	3.229	18.53	< **0.001**
	Treatment (Defoliated)	0.5002	2.196	0.0301
	Treatment (Isolated)	0.1492	0.692	0.4905
	Flower Position	0.0147	0.371	0.7111
	Treatment (Defoliated) * Flower Position	-0.2369	-4.097	<**0.001**
	Treatment (Isolated) * Flower Position	-0.0812	-1.214	0.2271
Nectar Guide lenght	Intercept	0.8407	8.246	< **0.001**
	Treatment (Defoliated)	-0.2943	-2.185	**0.0309**
	Treatment (Isolated)	0.0381	0.299	0.7657
	Flower Position	-0.0076	-0.323	0.7471
	Treatment (Defoliated) * Flower Position	0.1569	4.589	< **0.001**
	Treatment (Isolated) * Flower Position	-0.0056	-0.142	0.8876
Long Stamen lenght	Intercept	2.2162	43.877	< **0.001**
	Treatment (Defoliated)	0.3261	7.242	< **0.001**
	Treatment (Isolated)	-0.2754	-6.174	< **0.001**
	Flower Position	-0.0239	-2.158	**0.0329**
Short Stamen lenght	Intercept	0.9974	10.024	< **0.001**
	Treatment (Defoliated)	-0.1131	-1.794	**0.0754**
	Treatment (Isolated)	-0.4268	-6.912	< **0.001**
	Flower Position	-0.0187	-1.207	0.2300
Styles lenght	Intercept	1.7155	13.023	< **0.001**
	Treatment (Defoliated)	0.2411	2.586	**0.0109**
	Treatment (Isolated)	-0.0697	-0.762	0.4474
	Flower Position	0.0244	1.062	0.2903

ARTIGO 3

DOES CLONAL INTEGRATION AND RESOURCE DEPLETION MEDIATE VEGETATIVE TRADE-OFFS ON RAMETS OF *Eicchornia crassipes* (PONTEDERIACEAE)?

DOES CLONAL INTEGRATION AND RESOURCE DEPLETION MEDIATE VEGETATIVE TRADE-OFFS ON RAMETS OF *Eicchornia crassipes* (PONTEDERIACEAE)?

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ABSTRACT

Natural selection favours the allocation of finite resources to different functions maximizing fitness. In this sense, some functions may decrease whereas others increase when resources are limited, in a process called trade-off. However, a great variety of situations may obscure trade-off detection, as the ability of generating offspring by clonal growth that represents opportunities resource uptake, in clonal plants. The aim of this work was to evaluate if clonal integration and resource availability mediates biomass allocation patterns on *E. crassipes*, through a greenhouse experiment. We set ramets in clonal and isolated conditions, and with and without leaf blades, and compared relationship of the biomass proportion allocated to each vegetative organ. We found trade-off patterns of biomass allocation among vegetative organs and that under resource depletion, clonal integration may represent a way to stabilize biomass allocation patterns and may decrease trade-off importance. We discuss trade-offs and clonal integration as evolutionary strategies that allow plant persistence and improves plants fitness.

Key words: allocation theory; aquatic macrophyte; life-history strategies; reproductive strategies

1. INTRODUCTION

The allocation of resources to different plant parts is a central aspect of plant life histories and responses to environmental change. Each organism life depends on environmental resource and energy pools in order to realize their metabolic functions. These components are not unlimited, and so individuals are ought to allocate them to fulfil their most limiting needs on a given time and space (Abrahamsom, 1980). Optimal biomass partitioning theory predicts a higher allocation to the organ increasing the uptake of the most limiting resource for growth (Bloom, 1985), and life-history theory predicts a higher allocation to the organs limiting survival or reproduction, optimizing fitness (Stearns, 1992). Therefore, an individuals' success will depend on how the allocation of plant biomass to different parts or organs (i.e. roots, stems, leaves, flowers) relates to environmental resources. However, depending on how severe resource limitation is, this differential allocation may be biased towards some functions or organs, and the allocation to one function might cause the decrease in other, generating an allocation trade-off (Roff, 2002).

Trade-offs are well documented on the literature dealing with plant ecology and may be related to plant reproductive strategies (Abrahamsom, 1975; Thompson and Eckert, 2004; Coelho et al., 2005; Coelho et al., 2006; Cao and Worley, 2013). However, a great variety of situations may obscure trade-off detection (Roff and Fairbairn, 2007). In the case of aquatic plants, for example, the extensive ability of generating offspring by clonal growth (Santamaría, 2002; Barret, 2015), may represent an opportunity for new resource acquisition and/or storage organs generation (Grace, 1993). Furthermore, life-history theory predicts a higher allocation to the organs limiting survival or reproduction, optimizing fitness (Bazzaz et al., 2000). Clonality seems to be a key life-history trait for plants ecology (Eckert et al., 2016). In relation to reproductive strategies, the alternation between sexual and asexual reproduction is, sometimes, recognized as a bet-hedging strategy (Niklas and Cobb, 2017) in the sense that sexual reproduction is responsible for introducing genetic variability, what increases the probability of a species survival under suddenly local changes. On the other hand, asexual reproduction may eliminate some costs of sexual reproduction and can preserve well adapted genomes (Park et al., 2014; Stelzer, 2015; Chambers and Emery, 2016). Thus, clonality, via asexual reproduction, would allow a transgenerational fixation of well adapted phenotypes. This trait is strongly related to many other processes, as propagule dispersion (Santamaría, 2002; Barret, 2015), resource acquisition (Stuefer et al., 1994), sexual reproduction, and reproductive success (Thompson and Eckert, 2004; Mori et al., 2009; Demetrio et al., 2014), plant mating patterns (Charpentier, 2002; Vandepitte et al., 2013), pollinator behaviour (Liao and Harder, 2014), and population growth (Coelho et al., 2005; Schulze et al., 2012). Regarding trade-offs, clonality may also bias the biomass allocation patterns, since clonal plants may function as highly integrated units (Stuefer et al., 1996), leading to a modular comprehension of this plasticity in plants (de Kroon et al., 2005).

In this sense, the aims of this paper are i) to understand how clonality, *via* clonal integration, affects biomass allocation patterns on a widely distributed aquatic plant, ii) to verify if this trait mediates trade-offs among vegetative organs under control and impoverished resource pools conditions, and iii) to verify if clonal integration means an advantage for the ramets, causing the absence or the smoothing of the potential trade-offs in daughter ramets, generating a biomass allocation to all parts of the plant. We hypothesize that clonal integration will mask trade-offs between biomass allocation to different vegetative organs or, at least, generate a smoother relationship when compared to plants that was isolated from parental ramets.

2. MATERIALS AND METHODS

2.1 Study species

Eicchornia crassipes (Mart.) Sölms. is a free-floating macrophyte (Barret, 1980, Pott and Pott, 2000), that originally inhabited Amazon river basin (Pott and Pott, 2000). However, it has recently been recognized as an aggressive invasive species (Holm et al., 1977), reaching a worldwide distribution (Barret, 1989), mainly due to its ornamental value. Its constituted by a little stem from which leaves in a rosette organization and several fine roots appear (Pott and Pott, 2000). It also presents strong vegetative reproduction via rhizome sprouting and can rapidly cover great water-surface extensions (Watson, 1984).

2.2 Plant sampling

All plant material was collected from a great monospecific mat located at Represa do Funil. Plants were collected and previously washed in the field. We selected adult ramets (identified by the presence of newly produced sexual reproductive structures or their remaining - as old floral scapes) with no signs of foliar herbivory or diseases. We selected a total of 90 ramets that were put in plastic bags filled with some water to avoid root desiccation and took them to a greenhouse at Federal University of Lavras in order to carry the experiment.

2.3 Greenhouse experiment

We distributed the ramets among eighteen pots filled with 17 L of tap water. Ramets were left inside the greenhouse for a period of two weeks without any interference in order to allow an acclimation period. After this time we selected 36 ramets of similar size that were isolated in pots filled with 17 L of tap water. The ramets were cultivated until the production of asexual offspring for each of the selected ones. This first generation of ramets produced under greenhouse conditions are referred as “parental” from hereafter. Parental ramets remained attached to the ramets from which they derived until they also produced a generation of asexual offspring.

After this, parental ramets and their offspring were isolated, forming the basic unit of the experiment. These groups were placed in 36 pots filled with 17 L of tap water. After this, we set four treatments on these parental-daughter ramets groups. The first one, ‘isolation treatment’, consisted on nine daughter ramets that were experimentally split off from their parental ramets. The second one, ‘clonal treatment’, consisted on the maintenance of nine groups as originally conceived, with the daughter ramet attached to the mother ramet. The third one, ‘defoliation and isolation treatment’, consisted on nine daughter ramets that were experimentally split off from their parental ramets and also had all of their leaves removed at the beginning of the experiment, simulating a resource shortage. The fourth one, ‘defoliation treatment’, consisted on the maintenance of nine groups as originally conceived, with the daughter ramet attached to the mother ramet, but with the removal of all their leaves at the beginning of the experiment. The experiment was carried out for three months.

2.4 Plant biomass measurement

In the end of the experiment all plants were washed and too taken to the laboratory. Each ramet was separated in stem, petioles, leaf blades, and roots. These structures were put in individual paper bags and dried at 60ºC during 60 h or until we obtained a constant mass. After that, ramets parts mass was measured in a precision scale. In order to verify the effects of our treatments on biomass allocation patterns we first calculated the proportion of biomass allocated for each structure, in each individual ramet. This procedure was made by dividing the obtained mass of each ramet part by the ramet total biomass, what generated a proportion of biomass allocation invested in each of the measured parts.

2.5 Data analysis

All variables were tested for normality with Shapiro-Wilk tests. To evaluate the influence of resource depletion on *E. crassipes* biomass allocation patterns and its possible interactions with clonal integration, we applied GLM's with quasi-binomial distribution for all variables. For every model the biomass proportions allocated to each plant part were inserted, one at a time, as the response variable, and the biomass proportion allocated to the other plant parts treatment (isolated, clonal, and, defoliation and isolation, and defoliated condition), and their interaction term were inserted as a fixed variable. After that, we used a stepwise backwards variable selection procedure for each response variable. All analyses were carried on R Statistical Software (R Core Team, 2017).

3. RESULTS

For all measured traits the ‘defoliation’ treatment was the only one that affected biomass allocation patterns, and defoliated ramets that remained attached to their parental plants showed lower biomass in relation to the other treatments. The importance and direction of the interaction among the treatments and the patterns of biomass allocation was specific for each variable, with the ‘defoliation treatment’ being the only one that significantly changed the slopes of the relationships.

The biomass allocation proportion to stems did not changed with the proportion of biomass allocated to the shoot components of the ramets. Nor proportion of biomass allocated to leaf blades (F = 1.89, p = 0.1092), or proportion of biomass allocated to petioles (F = 1.96, p = 0.09) showed any effect on biomass allocation to stems.

The proportion of biomass allocated to root system decreased with the increase in all of other plant parts. The decrease, however, was more intense when the response variables were related to the shoot parts of the plant (Table 1), with the proportion of biomass allocated to stems showing a negative, but less intense effect (Table 1), and showing no interaction with the applied treatments. In relation to shoot components, proportion of biomass allocated to leaf blades and petioles showed a pattern of decreased with the increase in the proportion of biomass allocated to stems or roots (Table 1). However, for both cases, the allocation for roots showed a more intense effect on allocation for shoot parts (Table 1).

In relation to shoot parts, leaf blades and petioles, for both cases, the interaction term between the proportion of biomass allocated to each response variable and the ‘defoliation’ treatment was significant, revealing a smoother slope in the decrease of proportion of biomass allocated to leaf blades and petioles when compared to the clonal treatment (Table 1) (Fig 1A-B).

Leaf blades and Petioles did not follow the pattern of trade-off found for the other characteristics. These traits showed a positive and significant correlation, exhibiting mutual increases in proportion of allocated biomass in all treatments (Table 1) (Fig 1C).

4. DISCUSSION

Our results show that *E. crassipes* biomass allocation is mediated by trade-offs occurring between root and shoot parts, and these trade-offs are smoothed by clonal integration when the ramet remains linked to its mother plant. Additionally, stress seems to be a potential driver of biomass accumulation, since decreases in resources availability (represented in this study by the resource depletion caused by defoliation) may cause an alteration on how *E. crassipes* ramets allocate biomass to its vegetative organs.

Differences in allocation to structures, and so functions, are probably more important to ecological questions than differences in physiological mechanisms occurring at cellular or lower levels (Schwinning and Weiner, 1998), since biomass allocation is closely related to the survival strategy of plants (Xie et al., 2014). This may occur mainly because phenotypic plasticity allows a genome to modify growth and development in response to changes in the environment (Dorken and Barret, 2004), what may lead individuals that are adjusted to their habitats to show trade-offs related to environmental conditions (Gratani, 2014).

The existence of trade-offs at the ramet level for aquatic macrophytes traits are still largely debated on literature. Some works relate absence of trade-off relationships among plants traits like biomass allocation or measures of vegetative organs (Coelho et al., 2000; Alvarenga et al., 2013), while others found trade-offs for root and shoot allocation (Li et al., 2011; Pereto et al., 2016). In the cases were trade-offs are present, they are commonly linked to important aspects of plant fitness, such as nutrient assimilation by roots, and leaf investment (Bouma et al., 2005). This holds true for our data set, since we found that the major part of relationships among *E. crassipes* vegetative organs are mediated by trade-offs, except for the relationship between biomass allocation to leaf blades and petioles, that was positive.

This is clear pattern when resources acquisition strategies are considered. Clonal plants use these strategies in response to resource allocation among ramets and adjust to particular environmental changes (Stuefer et al., 2002). For the genus *Eicchornia,* previous works have shown that for rooted species, like *E. azurea*, the nutrient level is an important driver for plants biomass allocation, with plants located in lentic habitats showing a strong trade-off among vegetative characteristics. This is due to the lower water renewal in these kind of habitat, a characteristic found in our study, conducted in pots under greenhouse conditions. Water flow promotes water renewal (Wetzel, 2001), what avoids nutrient depletion in a variety of habitats colonized by aquatic plants. This absence of nutrient renewal through water movement may be of extreme importance for floating plants, because aquatic habitats tend to be highly homogeneous (Santamaría, 2002), and nutrients tend to descend on the water column, becoming unavailable for roots uptake.

In the case of *E. crassipes,* the stem is just the connective part between root and shoot parts, with the function of anchoring leaves and roots. Not surprisingly, the proportion of plants total biomass allocated to this organ was not different between resource levels, nor different clonal integration conditions. Root traits, however, are very important to evaluate the degree of plant adjustment to new environmental conditions (Xie et al., 2006), and changes in root morphology are commonly observed in aquatic plants, with roots being bigger in habitats that show higher stress levels as high plant density (Alvarenga et al., 2013), low nutrient levels (Pereto et al., 2016) or increasing in size in gradients of aquatic to terrestrial habitats (Li et al., 2011). In our data set, roots decreased in biomass when an increase in leaves biomass was observed mainly due to the resources limitation to what plants were submitted, being more pronounced in the situations were resources could not be transported from parent to daughter ramets.

In this sense, clonal integration, under severe resource limitation, may decrease the importance of trade-offs, allowing a more balanced root/shoot ratio during daughter ramets growth. Therefore, clonal integration is advantageous to the clonal growth of parents and offspring ramets as whole (Alpert and Simms, 2002), what is corroborated by the changes in the whole plant in a way that enhances the uptake of the most limiting resource when all ramets lie within a homogeneous patch of habitats (Wijesinghe and Hutchings, 1999). For our data, severed ramets showed a different response to clonal integration, when compared to those that remained intact, but not when isolated. This demonstrates the importance of clonal integration on translocating resources and allowing ramets plastic response, as occurred for *Hydrocotyle bonariensis*, in which severed ramets showed more variable responses to nutrient levels (Evans, 1992).

We conclude that biomass allocation patterns to vegetative structures are mainly determined by resource pools. These relationships may respond in different ways to clonal integration, being less pronounced when the ramet is attached to its mother ramet, what indicates that clonal integration is an enhancer of *E. crassipes* growth and vegetative investment. This information set basis as an important tool on aquatic plants management and control, and improves the knowledge on clonal integration evolutionary importance and its significance to plants life-strategies.

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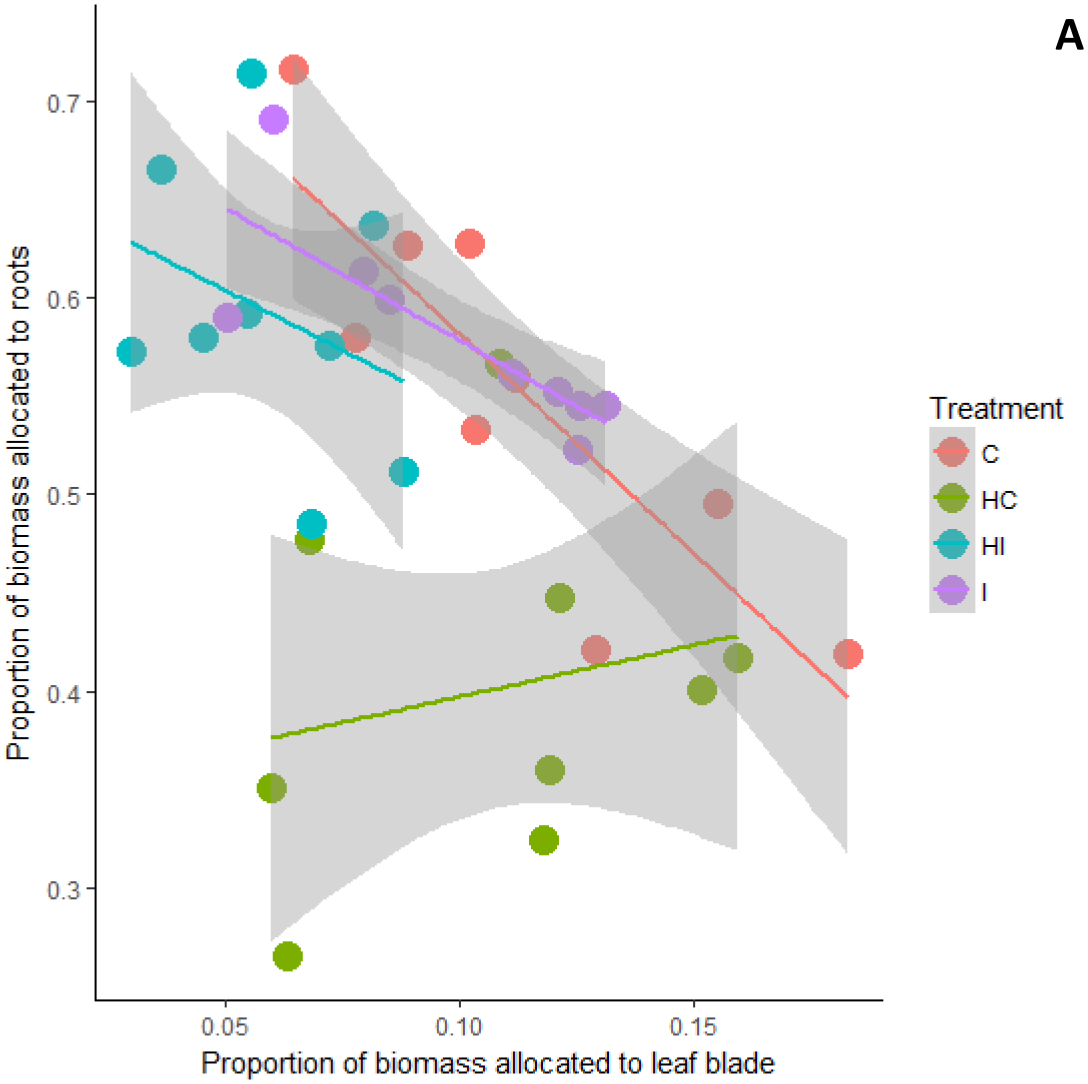
FIGURES AND TABLES

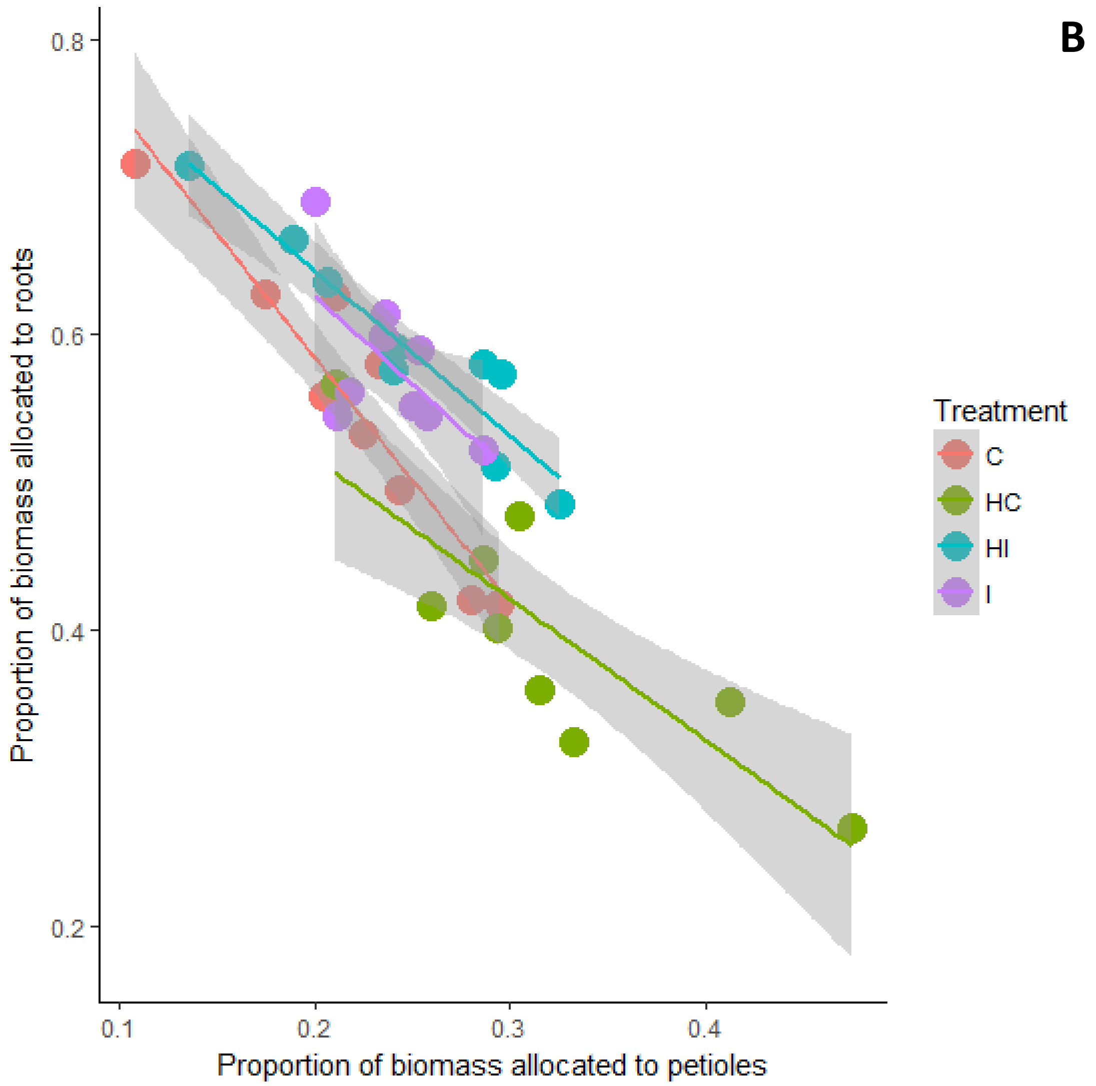
Table 1. Results of general linear models showing the relationships between the proportion of biomass allocated to each part of plant. Significative relationships are marked with *.

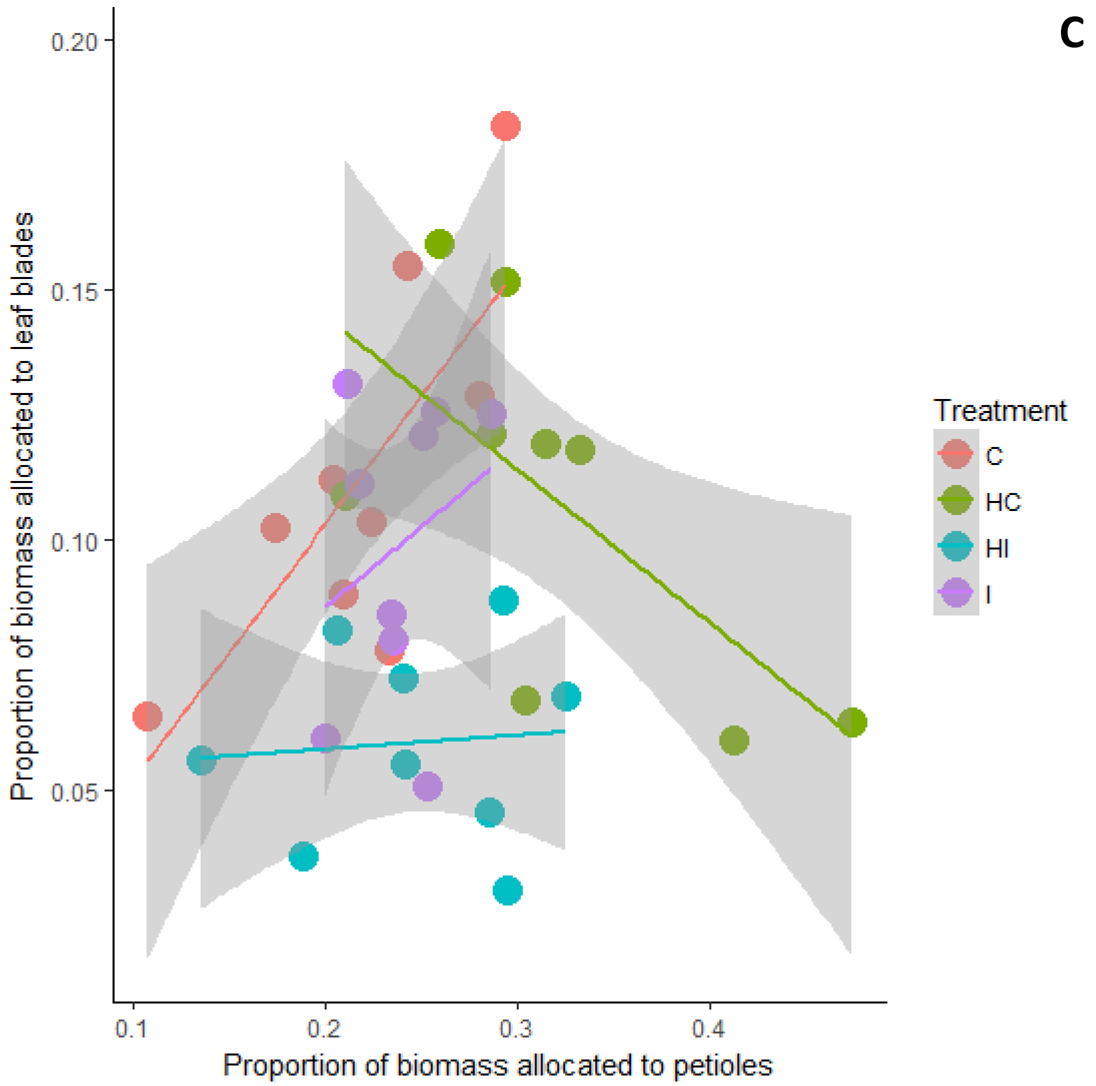
Y variable	X variable	Term	Estimate	t value	p value
Root biomass proportion	Stem biomass proportion	Intercept	0.6018	2.472	0.0191*
		Stem biomass proportion	-4.7915	-1.778	0.0853*
		Treatment (Defoliated)	-0.4651	-2.727	0.0104*
		Treatment (Isolated and Defoliated)	0.2632	1.649	0.1092
		Treatment (Isolated)	0.1160	0.779	0.4421
	Leaf blade biomass proportion	Intercept	1.2477	3.95	< 0.001*
		Leaf blade biomass proportion	-9.1477	-3.438	< 0.01*
		Treatment (Defoliated)	-1.8855	-4.29	< 0.001*
		Treatment (Defoliated and Isolated)	-0.5727	-1.298	0.2065
		Treatment (Isolated)	-0.3709	-0.803	0.4285
		Leaf blade x Treatment (Defoliated)	11.3403	2.997	<0.01*
		Leaf blade x Treatment (Defoliated and Isolated)	4.0868	0.725	0.474
		Leaf blade x Treatment (Isolated)	3.5611	0.849	0.403

	Petioles biomass proportion	Intercept	1.7691	7.303	< 0.001*
		Petioles biomass proportion	-7.0591	-6.638	<0.001*
		Treatment (Defoliated)	-0.8453	-2.459	<0.05*
		Treatment (Defoliated and Isolated)	-0.2198	-0.637	0.592
		Treatment (Isolated)	-0.2706	-0.478	0.63
		Petioles x Treatment (Defoliated)	2.8953	2.221	<0.05*
		Petioles x Treatment (Defoliated and Isolated)	2.31	1.612	0.1182
		Petioles x Treatment (Isolated)	2.1389	0.9	0.3759
Leaf blade biomass proportion	Petioles biomass proportion	Intercept	-3.3102	-7.009	<0.001*
		Petioles biomass proportion	5.5478	2.766	<0.01*
		Treatment (Defoliated)	2.2826	3.485	<0.01*
		Treatment (Defoliated and Isolated)	0.4207	0.563	0.57817
		Treatment (Isolated)	0.2458	0.225	0.823
		Petioles x Treatment (Defoliated)	-1.9822	-3.647	<0.01*
		Petioles x Treatment (Defoliated and Isolated)	-9.0158	-1.658	0.108
		Petioles x Treatment (Isolated)	-5.039	-0.436	0.666*

Figure 1. A) Relationship between the proportion of biomass allocated to roots and leaf blades of *E. crassipes* ramets in relation to levels of clonal integration and experimental defoliation. B) Relationship between the proportion of biomass allocated to roots and petioles of *E. crassipes* ramets in relation to levels of clonal integration and experimental defoliation. C) Relationship between the proportion of biomass allocated to leaf blades and petioles of *E. crassipes* ramets in relation to levels of clonal integration and experimental defoliation. C represents the clonal treatment, I represents the isolated treatment, HI represents the defoliated and isolated treatment and HC represents the defoliated treatment.




B
Proportion of biomass allocated to roots
0.8
0.6
0.4
0.2
0.1
0.2
0.3
0.4
Proportion of biomass allocated to petioles
Treatment
C
HC
HI
I


C
Proportion of biomass allocated to leaf blades
0.20
0.15
0.10
0.05
0.1
0.2
0.3
0.4
Proportion of biomass allocated to petioles
Treatment
C
HC
HI
I

CONCLUSÃO GERAL

A clonalidade apresenta-se, mais uma vez, como um aspecto chave da história de vida das plantas. Além de sua função reprodutiva óbvia, como geratriz de novos propágulos, ela também desempenha um importante papel na regulação da reprodução sexuada e na produção das estruturas florais. Esse mecanismo se dará, principalmente, devido à função de aquisição de recursos que muitos propágulos assexuados apresentam. Ao gerar uma estrutura análoga a uma rede de distribuição de recursos, a clonalidade, via integração clonal, permite um sucesso aumentado do genótipo, já que todos os rametes terão possibilidades de sobrevivência, crescimento e reprodução.

Os resultados deste trabalho demonstram que além da influência na quantidade da reprodução, a clonalidade está intimamente ligada à reprodução sexuada porque pode afetar o tamanho das estruturas sexuadas, como as flores. No caso de *Eicchornia crassipes* essa influência pode ser obscurecida graças à alta taxa de auto-fecundação. Entretanto, para espécies clonais exclusivamente autógamas, a integração clonal pode ser um forte mecanismo de aumento do sucesso de reprodução sexuada.

Além disso, outra parte importante dos processos de história de vida das plantas, a alocação de biomassa, está intimamente ligada à integração clonal. Isto porque um ramete ligado à planta mãe pode ter acesso a uma biomassa que não estaria disponível, caso essa conexão estivesse ausente ou fosse rompida. Neste caso, a clonalidade funciona como um atenuador de *trade-offs*, permitindo que várias funções recebam investimento de biomassa, tornando mais suaves as relações negativas entre a alocação de biomassa e/ou nutrientes para diferentes traços de história de vida. Um dos próximos passos seria a identificação de quais funções são privilegiadas pela biomassa produzida dentro do ramete e quais funções recebem biomassa "externa", advinda da mãe.

Concluo, por fim, que as respostas à integração clonal são, provavelmente, espécie-específicas. Entretanto, apresentados os resultados encontrados nesta tese, reforço a informação contida no corpo teórico no sentido de que a clonalidade é um caractere adaptativo, que oferece aumento do sucesso em habitats que, de alguma forma, são limitantes.