Universidade Federal de Uberlândia

Instituto de Biologia

RESISTÊNCIA INDUZIDA EM *Bauhinia brevipes* Vog. (FABACEAE) E INTERAÇÕES COM INSETOS DE DIFERENTES GUILDAS

Janete Ferreira Andrade

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Dissertação apresentada ao Instituto de Biologia da Universidade Federal de Uberlândia (UFU) como requisito para a obtenção do título de mestre em Ecologia e Conservação dos Recursos Naturais.

Orientador:

Prof. Dr. Jean Carlos Santos

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APROVADA em 23 de fevereiro de 2016

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Prof. Dr. Marco Antônio Alves Carneiro – UFOP

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Profa. Dra. Maria Cristina Sanches – UFU

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Prof. Dr. Jean Carlos Santos – UFU

Orientador

UBERLÂNDIA
Fevereiro - 2016

“Comece devagar,

porque a direção é mais importante que a velocidade...

Tente o novo todo dia:

o novo lado,

o novo método,

o novo sabor,

o novo prazer,

o novo amor,

a nova vida.

...O mais importante é a mudança,

o movimento,

o dinamismo,

a energia.

... Só o que está morto não muda.”

Edson Marques

Dedico à minha família:

À minha mãe Miria e aos meus irmãos Lú e Renato, que aqui estão comigo;
À minha linda sobrinha Heloisa, que acabou de chegar;
Ao meu pai Idalino e à minha irmã Bárbara, que já partiram...

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SUMÁRIO

1 RESUMO

Andrade, Janete Ferreira; 2016. Resistência induzida em *Bauhinia brevipes* Vog. (Fabaceae) e interações com insetos de diferentes guildas. Dissertação de Mestrado em Ecologia e Conservação de Recursos Naturais. Universidade Federal de Uberlândia. Uberlândia-MG. 58 p.

Interações como a herbivoria há o consumo de partes de tecidos vegetais por insetos herbívoros que promovem impactos no desempenho vegetativo e reprodutivo de plantas hospedeiras. Mecanismos de defesas induzidas como respostas de hipersensibilidade (RH) são observadas em diversas espécies e têm relação direta com a especificidade entre inseto herbívoro e planta hospedeira. No entanto, a capacidade de desenvolver RH varia de acordo com o genótipo e fenótipos de plantas hospedeiras e com distúrbios ambientais como o fogo. Assim, o objetivo geral do nosso estudo foi avaliar o desempenho de *Bauhinia brevipes,* a capacidade de desenvolver defesas induzidas contra o inseto galhador *Schizomyia macrocapillata* e as taxas de herbivoria por insetos de vida livre. Avaliamos essas interações no contexto das variações fenotípicas em desenvolver respostas de hipersensibilidade, e de distúrbios ambientais como o fogo. Assim, no primeiro capítulo, avaliamos o desempenho de plantas adultas e da progênie de acordo com os fenótipos de resistência (resistente e susceptível) aos ataques de *S. macrocapillata*. Para tal, avaliamos o desempenho vegetativo como arquitetura e o desempenho reprodutivo como número de sementes (componente feminino), o número de frutos formados (componente masculino) e a qualidade nutricional (macro e micronutrientes) em indivíduos resistentes e susceptíveis. Além disso, avaliamos o desempenho da prole em três estágios de desenvolvimento: sementes, plântulas e juvenis. Não houve diferenças nos desempenhos vegetativo e reprodutivo das plantas adultas, bem como na relação entre macro e micronutrientes e o fator resistência entre os fenótipos. Além disso, as taxas de germinação e investimentos em porção aérea e subterrânea na prole dos diferentes fenótipos foram similares. Acreditamos que a baixa incidência de *S. macrocapillata* sobre a população de *B. brevipes* contribuiu para o desempenho similar entre os fenótipos. Além disso, estratégias como tolerância à herbivoria devem ser analisadas nesse sistema inseto galhador / planta hospedeira. No segundo capítulo, avaliamos como as interações entre herbívoros e plantas hospedeiras, no contexto das defesas induzidas, como RH e defesas mecânicas como o acúmulo de silício em plantas com diferentes respostas ao fogo. Verificamos os indivíduos da população de *B. brevipes* apresentaram diferentes características quanto à incidência de fogo. As plantas que não rebrotaram apresentaram maiores taxas de herbivoria por insetos mastigadores e menores conteúdos de silício em relação às rebrotas. Por outro lado, as rebrotas foram mais atacadas por *S. macrocapillata*, mas exibiram maiores quantidades RH que àquelas plantas que não rebrotaram e em relação à estação anterior. Acreditamos que rebrotas de *B. brevipes* podem ser mais bem defendidas uma vez que alocam mais nutrientes, como nitrogênio para o crescimento e silício que reduz o desempenho de insetos mastigadores. As RH também podem ser aumentadas pela alocação de recursos pós-fogo o que levou às maiores taxas de defesas entre as rebrotas. Este estudo forneceu evidencias sobre o papel do fogo nas interações entre plantas hospedeiras e insetos de diferentes guildas e possíveis alterações no comportamento de insetos mastigadores.

Palavras-chave: Respostas de hipersensibilidade; insetos galhadores, insetos mastigadores, fogo, silício.

2 ABSTRACT

Andrade, Janete Ferreira; 2016. Induced resistance on *Bauhinia brevipes* Vog. (Fabaceae) and interactions with insects from different guilds. MSc. thesis. UFU - MG. 58 p.

In interactions such as herbivory the consumption of plant tissue by herbivore insects promotes several impacts on vegetative and reproductive performance of host plants. Defense mechanisms induced as hypersensitive response (HR) are observed in several species and is directly related to the specificity of herbivore insects and host plants. However, the ability to develop HR varies according to genotype and phenotype of plant hosts and with environmental disturbances such as fire. Thus, we aimed evaluate the performance of *Bauhinia brevipes*, the ability to evolve induced defenses against gall insect *Schizomyia macrocapillata* and herbivory rates by free-living insects. We analyzed these interactions in the context of phenotypic variation of resistance and environmental disturbances as fire. Then, in the first chapter, we evaluated the performance of mature plants and offspring according to the phenotypic resistance (resistant and susceptible) to *S. macrocapillata* attacks. Thus, we evaluate vegetative performance as architecture and reproductive performance as number of seeds (female component), the number of fruits (male component) and the nutritional quality (macro and micronutrients in leaves) on resistant and susceptible individuals. In addition, we evaluated the performance of the offspring in three stages of development: seeds, seedlings and juveniles. There were no differences in vegetative and reproductive performances of mature plants from resistant and susceptible phenotypes. Besides, there was no relationship between macro and micronutrients and the resistance factor. Germination rates and investments in shoot and root portion in the offspring of different phenotypes were similar. We believe that the low incidence of *S. macrocapillata* on the population of *B. brevipes* contributed to the similar performance between phenotypes. Moreover, strategies such as tolerance to herbivory should be analyzed in this gall insect / plant host system. In the second chapter, we evaluate the differences in responses to fire effects in relation to interactions between herbivore insects and host plants in and induced defenses (HR and silicon content). We detected an individual variation in response to fire as resprouting and non-resprouting plants in the population of *B. brevipes*. Non-resprouting plants showed higher rates of herbivory by chewing insects and lower silicon content in relation to resprouting. Contrasting, resprouting presented greater attack rate by *S. macrocapillata*, but exhibited larger amounts of HR than non- resprouting. We believe that resprouting of *B. brevipes* can be better defended, since allocate more nutrients such as nitrogen for growth and silicon which reduces the performance of chewing insects. HR can also be increased by the allocation of resources post-fire, which lead to the greatest defenses rates among resprouting plants. This study provided evidences about the role of fire in the interactions between host plants and insects of different guilds and possible changes in the behavior of chewing insects.

Keywords: Hypersensitive response; herbivory, galling insects, chewing insects, fire, silicon.

3 INTRODUÇÃO

Interações consumidor-recursos promovem o fluxo de energia através de cadeias alimentares e a estruturação de comunidades biológicas (COLEY & BARONE 1996, SCHMITZ 2008). Na herbivoria, por exemplo, há o consumo de partes de tecidos vegetais por agentes exofíticos e endofíticos que mesmo não removendo a planta hospedeira da população prejudicam a mesma (ZANGERL et al. 2002, SCHMITZ 2008). Nessa relação, as plantas hospedeiras sofrem inúmeros impactos no desempenho vegetativo e reprodutivo em virtude, principalmente, dos efeitos da perda de energia em biomassa (AGRAWAL & WEBER 2015) e da custosa manutenção de mecanismos de defesas (COLEY & BARONE 1996, BALLHORN et al. 2014).

A expressão da capacidade de defesas das plantas não é uniforme entre os indivíduos em uma determinada espécie (SHEN & BACH 1997). A resistência contra a herbivoria abrange variações genotípicas e fenotípicas na expressão e no desenvolvimento da capacidade de defesa (OLLERSTAM et al. 2003, HÖGLUND et al. 2015). Essas variações podem ocorrer na expressão de características ligadas à ontogenia das plantas (OLIVEIRA et al. 2012); na produção de compostos químicos secundários (AGRAWAL & WEBER 2015); na tolerância à ação de herbívoros (SHEN & BACH 1997); na concentração de silício no tecido vegetal (KORNDÖRFER & DEL-CLARO 2006) e no desenvolvimento defesas induzidas (KARBAN & BALDWIN 1997).

Defesas induzidas consistem em alterações físicas e químicas que ocorrem após o ataque de herbívoros, culminando na eliminação do agente indutor (FERNANDES, 1990, OLLERSTAM & LARSSON 2003). Este mecanismo também pode afetar o desempenho e o comportamento dos herbívoros através da resistência indireta que pode

incluir a atração de inimigos naturais (KARBAN & BALDWIN 1997, CHEN 2008), e consequente, redução na sobrevivência de insetos herbívoros (PASHALIDOU et al. 2013). Outro exemplo de defesa induzida consiste em respostas (ou reações) de hipersensibilidade (RH) (FERNANDES 1990, FERNANDES & NEGREIROS 2001, PASHALIDOU et al. 2013), as quais envolvem alterações bioquímicas, morfofisiológicas e histológicas na região adjacente ao tecido atacado. Essa reação culmina no acúmulo de compostos fenólicos, como as fitoalexinas, produção de metabólicos tóxicos e redução de oxigênio e conteúdo de água nos tecidos vegetais (FERNANDES & NEGREIROS 2001, PASHALIDOU et al. 2013) causando a morte do agente indutor. Apesar disso, estudos que abordam RH contra insetos galhadores ainda são escassos, tendo em vista a diversidade de plantas nos trópicos (FERNANDES et al. 2014).

Além dos fatores intrínsecos de defesas, condições ambientais, como o aporte de nutrientes disponível e distúrbios ambientais, também têm efeitos sobre o desempenho e susceptibilidade das plantas aos herbívoros (GARIBALDI et al. 2011). O excesso ou deficiência de macro e/ou micronutrientes nas folhas (BUTLER et al. 2012) ou nutrientes como silício (KORNDÖRFER & DEL-CLARO 2006) podem influenciar a capacidade defensiva contra a ação de herbívoros, aumentando a resistência mecânica e reduzindo a palatabilidade vegetal. Com relação aos distúrbios ambientais, o fogo além de ser um importante consumidor de biomassa vegetal, promove a estruturação de ecossistemas propensos a incêndios, como savanas neotropicais (BOND 2005) e auxilia na ciclagem de nutrientes do solo (GRAY & BOND 2015).

Após eventos de fogo, algumas espécies vegetais são capazes de produzir rebrotas pela ativação de gemas subterrâneas (CLARKE et al. 2013). Essas plantas sofrem alterações morfológicas e fisiológicas que podem ter efeitos sobre a qualidade

nutricional (VIEIRA et al. 1996), nas taxas de fotossíntese (WAN et al. 2014) e sobre a concentração de compostos de metabolismo secundários (LAVOIR et al. 2013). Essas alterações podem afetar os mecanismos de resistência contra insetos herbívoros e torná-las mais suscetíveis (VIEIRA et al. 1996, LOPES & VASCONCELOS 2011) ou resistentes (WAN et al. 2014, HOOD et al. 2015) ao ataque de insetos herbívoros.

Nesse sentido, *Bauhinia brevipes* Vogel (Fabaceae) destaca-se como um excelente modelo de estudo das interações entre insetos herbívoros e mecanismos induzidos de defesas. Essa espécie arbustiva típica do cerrado apresenta sistema subterrâneo bem desenvolvido, com capacidade de rebrotar após distúrbios como o fogo (VAZ & TOZZI 2003). É compartilhada por diferentes guildas de insetos como mastigadores, sugadores e galhadores, além de fungos endofíticos (SANTOS et al. 2008).

Bauhinia brevipes apresenta ainda alta capacidade de eliminar ovos do inseto galhador *Schizomyia macrocapillata* Maia (Diptera: Cecidomyiidae), através de respostas (ou reações) de hipersensibilidade, como já descrito para esse sistema, (FERNANDES & NEGREIROS 2001, SANTOS et al 2008, DETONI et al 2011) sobre as folhas de esta planta hospedeira (SANTOS et al., 2008). Nesse sistema, indivíduos resistentes de *B. brevipes* são capazes de localizar e eliminar as células induzidas antes da formação das galhas. Ao redor dos locais atacados se desenvolve uma marca amarelo-amarronzada (reação de hipersensibilidade) a qual consistirá na necrose tecidual da região atacada (FERNANDES et al., 2000, SANTOS et al., 2008).

Assim, o objetivo geral do nosso estudo foi avaliar o desempenho e as taxas de herbivoria (por mastigadores e galhadores) no contexto de defesas induzidas e distúrbios ambientais como o fogo. Especificamente, para o primeiro capítulo propomos a hipótese de que os indivíduos de *B. brevipes* mais resistentes ao ataque de *S.*

macrocapillata apresentam maior desempenho em termos de crescimento e reprodução, e produzindo uma prole mais vigorosa do que indivíduos susceptíveis. Para o segundo capítulo, propomos a hipótese de que o fogo altera as interações entre herbívoros e plantas hospedeiras, sendo os indivíduos de *B. brevipes* que rebrotam mais susceptíveis ao ataque de herbívoros mastigadores e galhadores, devido a menores taxas de defesa e oferta de recursos.

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5 CAPÍTULO 1

Performance of resistant and susceptible phenotypes to the attack by a galling herbivore

Manuscrito a ser submetido ao periódico *Plant Ecology*

Performance of resistant and susceptible phenotypes to the attack by a galling herbivore

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5.1 Abstract

Variations in plants' genotype and phenotype are expressed in ways to develop defensive ability against herbivory. Induced defenses are well-known mechanisms that affect herbivore insect's performance and foraging behavior. We aimed to evaluate the performance of *Bauhinia brevipes* according to phenotypes of resistance (resistant and susceptible plants) to attacks by *Schizomyia macrocapillata*. From the hypothesis that there is a positive relationship between resistance to *S. macrocapillata* and host plant performance because the resistance can have high adaptive value. We evaluated plant architecture, nutritional leaf quality (vegetative performance) and the capacity to compound fruits and seeds (reproductive performance) between groups of plants. The offspring performance was also evaluated in three stages of development: seeds, seedlings and juveniles. There were no differences in vegetative and reproductive performances of resistant and susceptible mature plants. Besides, there was no relationship between nutritional leaf quality and the resistance to *S. macrocapillata*. Offspring performance was equal in both phenotypes in all developmental stages. We believe that the low incidence of *S. macrocapillata* on the population of *B. brevipes* contributed to the similar performance between phenotypes. Contrasting, plant defenses are costly, and the resistant genotypes are favored when the probability of damage for insects is high. However, these genotypes can be disfavored when the probability of herbivore attack is low. Susceptible individuals even can over-compensate the damages by *S. macrocapillata* increasing leaves and branches. Moreover, strategies such as tolerance to herbivory should be analyzed in this gall insect / plant host system.

Keywords*:* Induced defense, phenotypic variations, *Bauhinia brevipes, Schizomyia macrocapillata*

5.2 Introduction

Galling insects affect plant performance by decreasing growth, reproduction and survivorship of their host plants (Fernandes 1987, Larson & Whitham 1991, Marini-Filho & Fernandes 2011). These effects occur mainly by the drain of considerable amounts of resources indispensable to the maintenance of the host plants (Larson & Whitham 1991, 1997, Compson et al. 2011). However, plants are not defenseless against such attacks as they can reduce the amount of herbivore damage by various defense mechanisms such as biochemical responses (Ollerstam & Larsson 2003). Defensive capacity of plants is directly related to genetic features and their interactions with the environment (Ballhorn et al. 2011) but it is not uniform among individuals in a particular species (Shen & Bach 1997).

Variations in genotype and phenotype of plants are expressed generally in how the defensive ability against herbivory is developed (Ollerstam & Larsson 2003, Holeski et al. 2012, Höglund et al. 2015). These differences may consist mainly in production of secondary chemical compounds (Laitinen et al. 2005), ontogeny and phenology of plants (Oliveira et al. 2012), indirect defenses as ant associations (Del-Claro & Marquis 2015), increased in the number of leaves and/or reproductive attributes after the insect damage (tolerance) (Shen & Bach 1997) and development of induced defenses that reduces the herbivore survivorship (Chen 2008, Pashalidou et al. 2013).This this type of defense requires significant intimate relationship between species involved and inductor agent life's history, mainly in galling insects and host plants systems (Fernandes & Negreiros 2001, Höglund et al. 2015).

Induced defenses are well-known mechanisms against herbivores (Chen 2008), and affect the herbivore's performance and behavior by attraction of natural enemies (Karban & Baldwin 1997, Chen 2008) or by elicitation of hypersensitive response

(Fernandes 1990, Pashalidou et al. 2013). It consists in a localized induced resistance that may be detected only in the immediately adjacent area to the site of attack (Larsson & Strong 1992, Fernandes & Negreiros 2001, Petzold-Maxwell et al. 2011, Pashalidou et al. 2013). The number of systems where such efficient reaction towards the insect herbivore is promptly elicited has been increased according detailed studies are being done. It has now being even reported in larger numbers in studies that demonstrate plants reactions against lepidopteran oviposition, for example (Bingham & Agrawal 2010). In this case, the plant elicits a reaction at the oviposition site that acts removing the herbivore eggs through a series of reactions which result in an attacked tissue's necrosis (Fernandes 1990, Pashalidou et al. 2013). Furthermore, is important to highlight antagonistic interactions tend to give damages to plants, not only by reduction of photosynthetic plant tissues, but also by high cost to produce defenses (Agrawal 1998). This spending of energy can affect the investment on flower and seed production, which can affect the offspring performance (Mothershead & Marquis 2000).

In the system *Bauhinia brevipes* Vogel (Fabaceae)-*Schizomyia macrocapillata* Maia (Diptera: Cecidomyiidae), host plant hypersensitive response to galls are very common (Fernandes & Negreiros 2001, Santos et al. 2008, Detoni et al. 2011) on the leaves of this host plant (Santos et al. 2008). *S. macrocapillata* galling success on *B. brevipes* is strongly influence by host plant stress by leaf fluctuating asymmetry (Santos et al. 2013), and by differences in protein concentration in plant tissues within resistant and susceptible phenotypes (Detoni et al. 2011), for example. In this interaction, resistant individuals are able to locate and eliminate the leaf cells induced by the galling insects before gall forming. At the attacked sites a necrotic spot develops around the galling site (hypersensitive response) preventing further development (Fernandes et al. 2000, Santos et al. 2008).

In this study, we provided further information about two different plant phenotypic performances in relation to the *B. brevipes* ability to elicit hypersensitive responses against *S. macrocapillata* attack. We believe in the hypothesis that a positive relationship between resistance and overall host plant performance, including offspring performance. Additionally, we examined if there are differences in leaf nutrient content (macro- and micronutrients) between resistant and susceptible phenotypes. The deficit or excess of leaf's nutrient can lead to plant stress and therefore, it can influence plant performance in its growth, reproduction and defensive ability against insect herbivores.

Herbivory is the main factor to limit plant performance and fitness. Therefore, we believe that resistant plants of *B. brevipes* have better performance, once that resistance to *S. macrocapillata* represents higher adaptive value in relation to susceptible plants, which suffer more damage. We addressed the following questions: (i) Are the resistant plants more successful in reproductive and structural (vegetative) terms? We expect that resistant plants show higher reproductive and structural performance due to higher defensive capacity and consequently low damages by *S. macrocapillata* (ii) Are the defensive ability and plant performance related to the nutritional quality of the individuals? We expect that resistant plants have more content of macro and micronutrients, which are related to defensive capacity of resistant plants (iii) Are the resistant plant's offspring more successful than the susceptible plant's offspring? We expect that there will be a differential investment in resistant plant's offspring, once those plants are more adaptive than the other one.

5.3 Material and Methods

Study area

The study was developed at the Estação Ecológica do Panga, a protected area located 30 km south of Uberlândia, State of Minas Gerais, Brazil (19º10'S and 48º24'W). The region is characterized by a Subtropical Climate with two well defined seasons: a dry winter (from May to September) and a rainy summer (from October to April). The average annual temperature is about 22°C, whereas the precipitation is 1,650 mm by year. The soils at the site are primarily red latosols, but it varies from moderately to strongly acidic (Lima et al. 1992). The vegetation formation comprises areas of different Brazilian Cerrado vegetation (Savanna) and forest formations (Ribeiro & Walter 2008). Specifically, the plant formations consist in "cerrado campo sujo" on the collect site, and all individuals was exposed on same light and water conditions.

Species of study

Bauhinia brevipes is a shrub species that grows to 3 meters tall, which distribution is around the states of Bahia, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Piauí, Rondônia, São Paulo and Tocantins (Vaz & Tozzi 2003). It is a deciduous species that loses its leaves between May and August. New leaves start to develop at the beginning of October, in concomitant period of increasing air humidity. It finishes in March when the rainy period ends. The flowering period covers the months from May to September with peak in July (Silveira et al. 2015).

Schizomyia macrocapillata Maia (Diptera: Cecidomyiidae) galls are red-colored, covered by trichomes, formed on the adaxial leaf surface, and occurring single or in coaslescent groups (Maia & Fernandes 2005). The oviposition occurs at the beginning of the rainy season (October), when leaves are young and still unfolded (Sá et al. 2009).

Gall development takes place from October to March, when eggs are laid. Gall survivorship is low, with more than 90% of the larvae being killed by hypersensitive responses (Santos et al. 2008).

Resistant and susceptible phenotypes

In March 2013 was performed a census in a population of 42 individuals of *B. brevipes* according to the plant's ability to elicit hypersensitive response to *S. macrocapillata*. All of these individuals showed height between 0.5 and 3 meters as criteria of inclusion. The Hypersensitive Response (HR) was characterized as a yellowish-brown color spot on the leaf surfaces (see Fernandes et al. 2000, Fernandes & Negreiros 2001, Petzold-Maxwell 2011 and Pashalidou et al. 2013).

The separation of phenotypes occurred according to the relationship: number of HR / (number of HR + number of galls). Thus, we classified the 12 plants with the highest amount of HR marks (ranged from 2 to 19) and the lowest formation of *S. macrocapillata* galls (ranged from 0 to 19) as "resistant plants". On the other hand, we classified the 12 plants with the lowest amount of HR marks (ranged from 0 to 5) and the highest formation of *S. macrocapillata* galls (ranged from 0 to 103) as "susceptible plants" ($U = 144$, $N = 24$, $P < 0.001$).

Plant performance

To determinate the structural and reproductive differences between phenotypes, we recorded the plant's height, crown diameter, number of branches, and number of leaves between the 12 resistant and the 12 susceptible host individuals (November 2013 to January of 2014). To estimate the *B. brevipes* reproductive performance, were recorded the proportion of inflorescence that produced fruits, and also the number of

seeds per fruit in the period from July to October of 2014, in the same individuals. According its fitness, plants was separate in female and male compounds as suggests Agrawal (1998). The female estimation of reproductive performance index was calculated by multiplying seed number by mean seed mass (Agrawal 1998). To estimate the male compound of reproductive performance, we performed the calculation: number of fruits / (number of fruits + mean number of bud flowers), to ensure the proportion of bud flowers that were fertilized in fact.

Nutritional analysis

To assess if the defensive ability to develop hypersensitive response and plant performance (vegetative and reproductive) were related to the nutritional leaf quality, we analyzed the nutrient content of the leaf tissues of the host plant individuals. To that, we randomly collected 20 fully expanded and healthy leaves from the middle of the canopy of 24 plants (12 for each phenotype) of *B. brevipes*. Tissues were dehydrated at 70°C for 48 hours and 10g from the dry biomass per plant was sub-sampled for the chemical characterization. We determined the following concentration of macro and micro-nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca); magnesium (Mg) and sulfur (S) as macro-nutrients, and boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) as micro-nutrients. All nutrients were analyzed in the Laboratory of Soils of Universidade Federal de Uberlândia (Uberlândia, Minas Gerais). We performed the analyses of nutrients according to the methods used by Santos et al. (2013). The nitrogen was analyzed by flow injection analysis, and phosphorus was determined calorimetrically; potassium was determined by an air–acetylene flame spectrophotometer; calcium, magnesium, sulfur, iron, copper and manganese were

measured by atomic absorption spectrophotometry; and boron was determined by the hot-water method.

Offspring performance

All the fruits were carefully bagged during the period of fruiting to ensure the future collection of seeds. This plant species has explosive seed dispersion (Vaz & Tozzi 2003). When all fruits were opened, we excluded the immature and predated seeds of the amount produced (from July to October 2013). The remaining seeds were grouped according to parental plant phenotype (resistant or susceptible). We evaluated the performance of offspring from resistant and susceptible plants in three different phases of germination process: seed stage, seedlings and juveniles, carrying out the following experiments. In addition, we verified that seeds of *B. brevipes* present mechanical dormancy. Then, in all procedures of offspring performance, we broke the mechanical dormancy by scarification of seeds on sandpaper.

1) Seed performance

To evaluate if the offspring (seed stage) of resistant plants would present higher performance compared to the susceptible plant's offspring we conducted experimental seed germination (October to December 2014). The set of seeds was surface sterilized by immersion for 10 minutes in sodium hypochlorite (1 mL per 100 mL of distilled water). Seeds from resistant plants (control and scarified) and seed from susceptible plants (control and scarified) was distributed the 25 seeds into plastic gerbox-type boxes (four replicates by treatment) to germinate on fine-grained vermiculite (~160 mL) moistened in distillated water up to the field capacity (~80 mL). The seeds were maintained in a incubator with mean temperature of 25°C degree and 12 hr light/12 hr

dark photoperiod under fluorescent white lamps (Ranal & Santana 2006). We adopted the radicle growth as a criterion for germination seed. Germination was monitored daily with germinated seeds removal. To measure the seeds performance we analyzed the following parameters of development: germination rate (G), mean germination time (t), mean germination rate's velocity (v), and synchronization index (Z) (Ranal & Santana 2006).

2) Seedling performance

To analyze the morphology and possible differences on development of seedlings from resistant and susceptible plants of *B. brevipes*, we conducted in October 2014 a seed germination procedure. We were designed it in four replicates of 10 seeds, all of them belonging to the resistant and susceptible plants. We used as substrate moistened paper towel rool with distilled water equal to two times the weight of the dry paper (Adapted from Zanotti et al. 2014). We distributed the seeds on two sheets of paper, it was covered with a third sheet, and forming a structure like a roll of paper. We packaged the rolls in plastic bags to prevent dehydration. It was maintained for 10 days in a incubator, with mean temperature of 25°C degree and 12 hr light/12 hr dark photoperiod under fluorescent white lamps (Pereira et al. 2015). After this period, we evaluated seedling features as cotyledons (position and thickness) and length of epicotyl, hypocotyl, root, and seedling dry weight.

3) Juvenile plants performance

To evaluate the performance (investment in growth) of juvenile plants from resistant and susceptible plants of *B. brevipes* we analyse the development of 40 juveniles from each resistant and susceptible plants (N = 80). Initially, we distributed

the seeds in vertical tubes with a mix of commercial substrate and vermiculite (110cm^3) located in plastic brackets (620x420x16mm). We cultivated these juveniles in greenhouse from November 2014 to February 2015 in a greenhouse under natural light conditions, temperatures ranging from 20 – 40°C degree, and intermittent irrigation. After 90 days, we removed all juveniles and measured the ratio of length from root and shoot; dry biomass ratio from root and shoot. Additionally, we measured the leaf area of three leafs from all juvenile individuals (N=240). We photographed these leaves at the laboratory and measured the leaf area using imageJ© software (Rasband 1997-2002) calibrated in centimeters.

Statistical analyses

We presented quantitative data as mean ± SE. Initially, we tested the assumptions of normality and homoscedasticity of all numerical variables. Following we performed a Mann-Whitney test to compared vegetative and reproductive performances; seed performance as rate (*G*), germination rate velocity (*v*), germination time (*t*) and synchronization index (*Z*); relationship between *shoot*: *root* ratio of seedlings and performance of juveniles as dry weight, length of root and shoot and leaf area (dependent variables) between resistant and susceptible plants. All comparisons were done in in environmental computational Systat 10.2 version.

5.4 Results

Plant performance

Susceptible individual plants were equal to resistant one despite height and number of leaves and branches. The differences between two phenotypes were no

statically significantly to height (t = -0.57, N = 24, P = 0.57), number of leaves (t = -.094, N = 24, P = 0.35) and number of branches (t = -1.94, N = 24, P = 0.24). The number of inflorescences (U = 074.5, N = 24, P = 0.88), fruits (U = 66.5, N = 24, P = 0.74), and seeds (U = 69, N = 24, P = 0.85) also did not differ statistically between susceptible and resistant phenotypes. Male (U = 67.5, N = 24, P = 0.78) and female (U = 71, N = 24, P = 0.95) compounds of plant fitness was not statically significant between resistant and susceptible plants. Regard to leaf nutrient these plant groups did not differ in any of their leaf contend of macro and micronutrients as see on Table 1.

Offspring performance

Overall, seeds of resistant and susceptible individuals presented germination rates near 100%. No statistical difference was found between the two groups and no statistically significant difference was also found in the velocity of seed germination rate, germination time, and synchronization index (Table 2).

Seeds of *B. brevipes* presented epicotyl were well developed and foliates and photosynthetic cotyledons above substrate, presenting epigeous germination. Seedlings of different groups of parent plants (resistant and susceptible) presented the same dimensions, the hypocotyl, root and dry weight of seedlings did not differ statistically between the resistant and susceptible plants (Table 2), but the length of the epicotyl was significantly larger in resistant than susceptible plants ranging from 12.76±0.81 mm in susceptible to 18.23±0.99 mm in resistant plants (t = 4.25, N = 74, $P < 0.001$).

Juvenile plants from resistant and susceptible phenotype show formation of xylopodya, an underground structure reserve. Juveniles plants showed differential investment between lengths of under and below ground portions ranging from 0.86±0.03 in individuals of susceptible plants to 0.96±0.03 in individuals of resistant

plants (t = 2.03, N = 80, *P* = 0.04). However, the dry biomass ratio (root: shoot) not differs between juveniles from resistant and susceptible plants (t = 1.53, N = 80, *P* = 0.35). As well as, leaf area also have no difference between juvenile plants from susceptible and resistant plants (t = -1.184, N = 80, *P* = 0.24).

5.5 Discussion

We found significant differences in elicit of hypersensitive responses and in the number of galls consequently between resistant and susceptible plants. Despite the marked difference in the defense rate, and consequently number of galls, and opposed to our expectations, the resistant and susceptible phenotypes showed an equal reproductive, vegetative and nutritional performance. Furthermore, we did not find substantial differences between the two phenotypes on offspring performance.

In general, these founds can be explained by the low density of galls in the leaves of all analyzed plants compared to previous years. Santos et al. (2008) recorded a variation of number of *S. macrocapillata* galls on *B. brevipes* leaves, which was higher in 1999 (22.5%) than in 2004 (6.64%). Even the smallest concentration of galled leaves recorded in 2004 was almost 20 times greater than the record of this study. Fluctuations on size population can explain the low index of attack by *S. macrocapillata* on *B. brevipes* (Cornelissen et al. 2011), and the low impact on all host plants which consequently show similar performance between the two analyzed phenotypes.

Damages from *S. macrocapillata* on *B. brevipes* can have local and not widespread effects on individuals. It is possible due to a low number of galls concentrated in a few branches or individuals. There was not a significantly effects on the individual or on population performance. Moreover, the intimate relationship

between galling insect and host plant is frequently associated to insect preference to oviposit, which creates conditions for better performance of galls and larger survivorship rate (Santos et al. 2008). As discussed by Agrawal (2005) induced defense's mechanism is only elicited by determinated organisms, as *S. macrocapillata* on leaves of *B. brevipes*, which is the most dominant specie of insect galling in this system.

Plant performance depends on a several environmental and biological conditions that influence the plant quality and resources allocations. Previous studies showed that there are increases on secondary compound after simulated herbivory on *B. brevipes* (Cornelissen & Fernandes 2001). This idea combined with the super host capacity of *B. brevipes* provides evidences that attacks by insect's guild added the incidence of *S. macrocapillata* may, synergistically, affect the overall plant performance. It is possible not only by reduction of photosynthetic plant tissue, but also by high cost to produce defenses (Agrawal 1998). Furthermore, is very known that defenses are costly; thus the resistant genotypes are favored when the probability of insect damage is high, but that these genotypes pay a cost for resistance and are disfavored when the probability of herbivore attack is low (Shen & Bach 1997, Strauss & Agrawal 1999, Agrawal 2005, Mundim et al. 2012). In this way, considering that susceptible plants of *B. brevipes* show similar performance as resistant plants. In addition, we believe that these plants invest more resources in defenses, despite of growth and reproduction. On the other hand, susceptible plants may be developing other ways to compensate the herbivore damage.

Similar concentrations of macro and micronutrients in leaves from resistant and susceptible plants of *B. brevipes* may have a direct relation to nutritional quality of the plants. It was similar between plant groups, and may have effects on capacity of

growing and productivity of plants. In this study, plants of *B. brevipes* were sampled in a relatively small area and uniform soil features, which may explain the low variation in concentration of nutrients available in both plant groups. Besides, the soil nutritional intake is actually low in savanna species, which may be lead to similarities in nutritional concentrations between resistant and susceptible plants (Mundim et al. 2012).

We also observed several similarities between offspring from resistant and susceptible phenotypes. Over the three stages of development we observed similar investments, both in germination and seed viability, as well as further investments in shoot parts and root. A possible explanation for the similarity in plant performance between both phenotypes consists in the facte that the susceptible individuals can over-compensate the damage by *S. macrocapillata*. We observed a similar pattern in susceptible individuals, which presented the double of leaves than resistant individuals in 2013 and 2014, presenting 1.20-fold and 2.13-fold leaves respectively. In this sense, is expected that plant performance could be compensated by reduction of herbivory effects. Several host plant-herbivores system insect shows strong investment of resources in growing and reproduction which regarded as important event of plant genotype adaptation (Shen & Bach 1997, Bingham & Agrawal 2010). In *B. brevipes*, susceptible individuals to galling attack produced more leaves, branches, and inflorescences and the germination seed rate was almost 100%, similarly to resistant plants.

This system of study has several details related to different interactions among this species and herbivore guilds sharing the same resources. As future directions, we suggest field studies that can isolate the effects of different herbivores on the plant fitness and defense mechanisms. This study showed a clear difference in the incidence rate of *S. macrocapillata* galls and defense rate by HR in *B. brevipes*. Besides, it gave

us the idea about tolerance to herbivory, and due that an equal plant performance between phenotypes. *Bauhinia brevipes* is super-host specie of several herbivores attackers. It shows from the simplest to the most sophisticated defense mechanisms, and a possible trade-off between resistance to their manly insect galling and plant performance.

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5.7 Tables

Table 1: Comparison of macro and micronutrients concentrations (mg kg−1) in leaves of resistant and susceptible plants of *Bauhinia brevipes* (Fabaceae) (Mean ± SE). The use of the Student's t-test or the Mann–Whitney U-test was based upon normality tests.

	Nutrients	**Resistant**	**Susceptible**	**Statistics**	***P*-value**
Macronutrients	Nitrogen (N)	12.8±0.35	12.64±0.36	$t = 0.309$	0.76
	Phosphorus (P)	0.30±0.03	0.29±0.04	$t = 0.162$	0.87
	Potassium (K)	6.5±0.47	5.70±0.40	$U = 95.5$	0.17
	Calcium (Ca)	12.4±1.85	14.28±1.75	$t = -0.89$	0.38
	Magnesium (Mg)	2.09±0.21	2.56±0.17	$t = -1.72$	0.09
	Sulfur (S)	0.46±0.01	0.50±0.02	$t = -1.07$	0.29
Micronutrients	Boron (B)	28.60±2.13	34.19±2.82	$t = -1.57$	0.12
	Copper (Cu)	2.25±0.22	2.47±0.34	$t = -0.54$	0.58
	Iron (Fe)	195.52±16.47	198.35±10.72	$t = -0.14$	0.88
	Zinc (ZN)	78.64±7.71	71.79±6.52	$t = -0.02$	0.98
	Manganese (Mn)	8.88±0.53	8.9±0.56	$t = 0.67$	0.50

Table 2: Comparison of seed and seedlings traits between resistant and susceptible individuals of *Bauhinia brevipes* (Fabaceae) (Mean ± SE). The Mann–Whitney U-test was based upon normality tests. Germinability rate (*G*), germination rate velocity (*v*), germination time (*t*) and synchronization index (*Z*).

Experiment	**Variables**	**Resistant**	**Susceptible**	**Statistic**	**df**	***P*-value**
Seeds performance	*G*	97.0±1	97.5±1.05	*U*= 28.5	14	0.68
	V	1.60±0.62	1.94±0.73	*U*= 26.5	14	0.56
	T	3.52±1.42	2.49±0.86	*U*= 37.5	14	0.56
	Z	0.38±0.13	0.21±0.07	*U*= 41.5	14	0.31
Seedlings performance	Epicotyl (mm)	18.23±0.99	12.76±0.81	*t*= 4.25	72	<0.001
	Hypocotyl (mm)	3.85±0.11	3.65±0.10	*t*= 1.25	72	0.21
	Root (mm)	24.71±1.22	23.52±1.66	*t*= 0.57	72	0.56
	Weight (mg)	0.027±0.001	0.027±0.001	*U*= 719	72	0.71
Juveniles performance	Root: Shoot ratio length	0.96±0.03	0.86±0.03	*t* = 2.03	78	0.045
	Root: Shoot ratio weight	1.62±0.08	1.47±0.06	*t* = 1.53	78	0.35
	Leaf area	7.26±0.29	7.81±0.35	*t* = -1.184	78	0.24

6 CAPÍTULO 2

Fire mediated herbivory and plant defense in a neotropical shrub

Manuscrito a ser submetido ao periódico *Oecologia*

Fire mediated herbivory and plant defense in a neotropical shrub

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6.1 Abstract

Changes in morphologic and physiologic features after fire events become plants more attractive to herbivores, as chewing and galling insects. We aimed to evaluate the fire effects on plant-insect interaction and development of induced defenses in plants of *Bauhinia brevipes*. We hypothesized that resproutings are more susceptible to attack by chewing and galling insects, due the lower defense rates and resource availability. Thus, we analyzed herbivory rate by chewing insects, attack rate by *Schizomyia macrocapillata*; hypersensitive response (HR) rate and silicon content in resprouting and non-resprouting. Non-resprouting plants demonstrated higher rate of herbivory by chewing insects and lower silicon content in relation to resprouting. Contrasting, resprouting presented greater attack rate by *S. macrocapillata*. However, they exhibited larger amounts of HR than non- resprouting. Additionally, within resprouting the HR rate was greater in 2015 than 2014. We believe that resproutings of *B. brevipes* can be better defended, since allocating more nutrients such as nitrogen which increases the growth and productivity. Silicon also has been allocated to plant tissues, which reduces the chewing insect performance and behavior. Hypersensitive response was increased by the allocation of resources post-fire, which lead to the greatest defenses rates to *S. macrocapillata* among resprouting plants. This study provided evidences about the role of fire on interactions between host plants and herbivore insects from different guilds. Increase on hypersensitive response and silicon content possibly change the behavior of herbivore insects.

Keywords: Resprouting, induced defenses, gall, silicon, *Bauhinia brevipes*

6.2 Introduction

Herbivores and fire are important plant biomass “consumers” and perform key roles in ecosystem structure (Bond 2005). In plant - herbivore insects interactions, for example, the activity of free-feeding (chewing-leaf and suckers) or endogenous (gall-inducers and miners) insects promotes the input of energy in biologic systems (Schmitz 2008). Therefore, fire is a singular source of disturbance driving changes in ecosystems structure of many terrestrial environments, and particularly in savannas (Bond & Keeley 2005, Gray & Bond 2015).

Fire is one of the most important features of the Brazilian cerrado (Coutinho 1977). It is a crucial factor for the maintenance of the structure, biodiversity and functioning of this ecosystems (Durigan & Ratter 2016). However, the human occupation at the cerrado region has changed the natural fire regime (intensity and frequency) with strong consequences on the structure and composition of vegetation (Miranda et al. 2002). The fire regime changes landscape from prone-fire ecosystems (as savannas) (Durigan & Ratter 2016). Increase in fires frequency, for example, lead to the expansion of open fields over wood vegetation (Setterfield et al. 2010). In contrast, it suppression for long periods leads to biodiversity losses with the replacement of savanna by forest formations, as in Australia (Scott et al. 2012) and Brazilian cerrado (Pinheiro & Durigan 2009).

Nevertheless, fire disturbance provides ways of ecological renewal by plant regrowth (Furley et al 2010), replacement of fauna (Frizzo et al. 2011) and changes in the amount of surface soil nutrients (Gray & Bond 2015). Large amounts of nutrients are released to the atmosphere during biomass burning, while the remaining supply is not readily available to plants (Debano & Conrad 1978). However, nutrients such as silicon do not have a gaseous form, which presents anti-herbivory action (Korndorofer

& Del-Claro 2006, Massey et al. 2006) and can be directly absorbed from the ground (Dias et al. 2014).

Resprouters are plant species that are able to regrow quickly after a disturbance by the activation of dormant vegetative buds after severe disturbances such as fire or grazing (Clarke et al. 2013). These plants (resprouting) tend to have better nutritional quality (Vieira et al. 1996), increase on photosynthesis rate (Wan et al. 2014) and variations in secondary compounds as terpenes and flavonoid concentration (Lavoir et al. 2013). Physiological and morphological changes on plant features can affect resistance against herbivorous insects and make it more susceptible to several insect feeding-guilds after fire (Stein et al. 1992, Vieira et al. 1996, Lopes & Vasconcelos 2011).

Thus, in the context of fire mediating interactions we highlight the neotropical shrub *Bauhinia brevipes* Vogel (Fabaceae) as a very good model of study. The species is typical cerrado shrub with subterraneous system (xylopodia) and capacity of regrow after fire (Vaz & Tozzi 2003). Besides, this species is attacked by several herbivores including many free feeding insects, fungal pathogens and at least seven galling species (Santos et al 2008, Silveira 2015). *Bauhinia brevipes* has the capacity of elicit induced responses to major galling herbivore, *Schizomyia macrocapillata* Maia (Diptera: Cecidomyiidae) by hypersensitive response (HR) (Santos et al. 2008, Detoni et al. 2011).

Several studies have reported the role of fire in promoting herbivory (e.g., Stein et al. 1992, Vieira et al. 1996, Kay et al. 2007, Lopes & Vasconcelos 2011) or on its reduction (Wheeler & Ordung 2006, Wan et al. 2014, Hood et al. 2015). However, few studies have evaluated the effects of fire on herbivore damage (Stein et al. 1992, Vieira el at. 1996, Lopes & Vasconcelos 2011) or plant induced defenses in species of

Brazilian cerrado. Thus, we have provided for the first time information about fire effects on interactions between a neotropical shrub and insect herbivores (generalist chewers and a specific gall-midge). It includes defense mechanisms such as hypersensitive response and silicon content in the leaves.

We hypothesized that resprouting plants have higher attack rate (by chewing-leaf and gall-midges) than non-resprounting ones due the higher photosynthetic capacity (Wan et a. 2014), growth rate (more foraging and oviposition sites) and less defenses against herbivory (Vieira et al. 1996, Lopes & Vasconcelos 2011). Besides, we believe that resprouting plants have lower content of silicon, which is an important cell wall component that prevents herbivores damage (Korndörofer et al 2010). Thus, we addressed the following questions regarding the effects of fire on plant-herbivore interactions: (i) Are there differences in physiological and morphological features in leaves between resprouting and non-resprouting plants? (ii) What is the most attacked group of plants by chewing insects and *S. macrocapillata:* resprouting or non-resprouting? (iii) Does fire change the capacity to develop induced defense as hypersensitive response? (iv) Is silicon related to herbivory by chewing insects and *S. macrocapillata* attacks, in resprouting and non-resprouting plants?

6.3 Material and Methods

Study area

Reserva Ecológica do Panga is a private protected area located 30 km south of Uberlândia, state of Minas Gerais, Brazil (19º10'S and 48º24'W). The region is characterized by a Subtropical Climate with two well-defined seasons: a dry winter (from May to September) and a rainy summer (from October to April). The average annual temperature is approximately 22°C, whereas the precipitation is 1,650 mm by

year. The soils at the site are primarily red latosols, but it varies from moderately to strongly acidic (Lima et al. 1992). The vegetation formation comprises areas of different Brazilian Cerrado vegetation (Savanna as vegetation) and forest formations (Ribeiro & Walter 2008), though over 70% of its area is covered by cerrado *sensu stricto* (Cardoso et al. 2009).

Species of study

Bauhinia brevipes is a shrub species that grows to 3 m tall, which is spread in the states of Bahia, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Piauí, Rondônia, São Paulo and Tocantins (Vaz & Tozzi 2003). It is a deciduous species, which the leaves fall between May and August. New leaves start to develop in the beginning of October, concomitant period of increasing air humidity, and finish in March when the rain ends. The flowering period happens between May to September with peak in July (Silveira et al. 2015). This shrub is shared by various guilds of herbivores, from galling insects (seven types) to leaf-chewing and branch-sucking insects and endophytic fungi (Santos et al. 2008). *S. macrocapillata* is the main gall maker of *B. breveipes* (Santos et al. 2008) and forms red-colored structures with hairiness on the adaxial leaves surface of the host plant (Maia & Fernandes 2005). Gall development takes place from October, when eggs are laid, until March and has almost 90% of mortality by hypersensitive response (Santos et al. 2008). This defensive mechanism is a little yellowish-brown color spot on the leaves' surfaces (see Fernandes & Negreiros 2001) and consists of a series of biochemical and anatomic changes that result in necrosis of the attacked tissue.

Sampling

Reserva Ecológica do Panga is a protected area and since its creation in 1986 it has been partially burned in 1992, 2003, 2006 (Lopes & Vasconcelos 2011) and 2014 due to human activities surrounding the reserve. In early October 2014, an accidental fire consumed the fine fuel of the litter and herbaceous layer affecting all vegetation's types of approximately 80% of this area.

To test all sets of predictions of this study, we used two groups of *B. brevipes*: the "resprouting" plants with 27 individuals and "non-resprouting" ones with 31 individuals. All of these plants were distributed evenly in the same site affected by fire, submitted to similar soil, vegetation (cerrado campo sujo), light and topography features. Resprouting plants lost the main branch and developed sprouts from the underground system (Clarke et al. 2013), while the non-resprouting ones did not develop any response to fire and reaming whit principal branches. In addition, it is important to highlight that the fire event occurred in the beginning of October 2014. This period was concomitantly to natural season of leaves regrowth, and then the collected leaves were the same age.

Resprouting and non- resprouting plants features

To evaluate if there were differences in trait plant between resprouting and non-resprouting plants of *B. brevipes* we analyzed the index SPAD of chlorophyll (SPAD unities), an indirect estimate of chlorophyll content as a physiological feature. Chlorophyll production is a crucial factor in photosynthesis process and its production depends on nitrogen allocation to leaves (Munhoz-Huerta et al. 2013). Furthermore, we used leaf length (cm) and leaf area (cm^2) as morphological feature.

We measured the index SPAD of chlorophyll during four months (from December 2014 to March 2015) from five randomly selected leaves per plant (fully expanded). We used a hand-held Chlorophyll Meter (SPAD 502 Plus, Konica Minolta). The index SPAD was used as an indicator of the photosynthetic capacity of resprouting and non-resprouting plants and it determines whether nitrogen allocation to leaf was affected by fire (Gaborcik 2003, Munhoz-Huerta et al. 2013). To analyse the morphological features, we randomly collected 870 healthy leaves (N=15 per plant) in January 2015. We photographed this set of leaves at the laboratory and measured leaf length from lamina tip to the intersection of lamina and petiole along lamina and the leaf area using imageJ© software (Rasband 1997-2002) calibrated in centimeters.

Herbivory by chewing insects and gall attacks

To evaluate what plant group has more attack rate by insect herbivory (resprouting or non-resprouting plants) and if the physiologic and morphologic features of these groups of plants are related to herbivory, we analyzed herbivory rate and total attempts of attacks by *S. macrocapillata* (number of gall as a successful oviposition + HR marks as attempt of oviposition) in both groups of plants. We collected leaves and galls of *B. brevipes* in January 2015, when the natural regrowth of leaves had already been completed and galls were established.

To estimate the plant damages caused by chewing insects, we randomly collected 1,740 leaves (N=30 per plant) completely extended photographed and analyzed them using imageJ© software (Rasband 1997-2002). Leaf damage by chewing insect was measured as: 1) the proportion of sampled leaves with herbivore damage, 2) the actual average leaf area removed (cm^2) and 3) removed leaf area percentage (Adapted from Leckey et al. 2014).

Regarding the damage caused by insect galls, we carried out a census in the same set of *B. brevipes* individuals (N=58 plants) collecting all galled leaves, and accounted the number of marks of HR with a hand counter. In the laboratory, we analyzed these leaves and recorded the proportion of sampled leaves with galls, the number of larval chambers of galls of *S. macrocapillata*.

Hypersensitive response and fire factor

We selected a subgroup of resprouting to evaluate if fire changes the capacity of *Bauhinia brevipes* elicit induced defense, as hypersensitive response against *S. macrocapillata*. These plants had the same set of parameters that we took over 2014 (before fire) and it was compared to the parameters collected over 2015. It consists of features related to gall incidence, such as number of galls, HR marks, and total attack attempts, which were analyzed as described in the previous section. In addition, we estimated *B. brevipes* resistance to *S. macrocapillata* following the relation: HR marks / (HR marks + total of galls) *100.

Silicon content analysis

To evaluate if silicon concentration was related to leaf chewing damage rate and *S. macrocapillata* attacks in resprouting and non-resprouting plants, we performed an analysis of leaf silicon (Si) concentration in these groups of plants of plants5. To do so, we took the same set of leaves used in the analysis of herbivory rate (30 leaves per plant from 58 individuals). Initially, these leaves were submitted to a complete extraction of water content for72 hours an average temperature of 40°C and it was milled in the Willey mill type. Finally, we determined the leaf Si concentration according to the

methods proposed by Korndörfer et al (2004) at the Laboratory of Fertilizer of Universidade Federal de Uberlândia (Uberlândia, Minas Gerais).

Statistical analysis

To analyze the physiological differences resprouting and non-resprouting after fire we performed a procedure of repeated measures ANOVA (Analyzes of Variance) in the GLM (Linear General Models). We analyzed the index SPAD of chlorophyll (dependent variable) in resprouting and non-resprouting plants (factor), using type III sums of squares. The morphologic differences between resprouting and non-resprouting plants (independents variables) were evaluated by tests of comparison of means such as Mann-Whitney for leaf area and Student-t test for leaf length (dependents variables).

To investigate if resprouting presents a higher herbivore rate we performed a test of Mann-Whitney. Both groups of plants' averages were compared related to herbivory rate and total attempted of attack by *S. macrocapillata* (dependent variables), and plants groups (independent variables). Besides, we performed Spearman rank correlations among leaf area, leaf length, herbivory rate and attacks of *S. macrocapillata* for each resprouting and non-resprouting group. In addition, in a subgroup of 25 individuals of resprouting plants, we evaluated whether the capacity of elicit hypersensitive response changed with fire. To do so, a Wilcoxon test was performed considering HR marks before and after the fire event.

We examined the silicon content effects on chewing-leaf insects and gall midges in resprouting and non-resprouting plants by ANCOVA (Analyzes of Co-variance) performed in GLM. Herbivory rate and attacks of *S. macrocapillata* were used as dependent variables, fire (resprouting and non-resprouting plants) was used as factor and silicon content was used as co-variable. Silicon effects, herbivory rate by chewing-

leaf and gall attacks had co-linearity analyzed by a model of linear regression. Before statistical analysis, we undertook a Johnson transformation on herbivory rate, which was transformed into normality using the Z family of distributions. Silicon content rate was log-transformed (*Logx*). We performed the set of analyzes in Minitab 17.1.0 software (version 17.10 Inc., State College, PA).

6.4 Results

Overall features

In general, aspects of the architecture of the groups of plants were remarkably different, mainly because resprouting plants lost their main branch and consequently its crow. These plants had an average of six resprouts with 25 internodes, reaching an average of 80 cm in height and 187 to almost 7,000 leaves. On the other hand, non-resprouting plants retained the crown, with an average diameter of 120 cm; seven branches subdivided em145 internodes with around 1,000 leaves.

Physiologic and morphologic features

Resprouting and non-resprouting plants also show several differences regarding physiologic and morphologic features. The variations in chlorophyll occurred between groups over time, with tendency of increase in chlorophyll between measurements times (Fig. 1). However, the group of plants was not related to time, once both resprouting and non-resprouting plants showed the same evolution in chlorophyll content over time (ANOVA repeated measures for chlorophyll: Plant Group, $F_{1,224} = 11.55$, $P = 0.001$; Time, $F_{3,224} = 4.86$, $P = 0.003$; Fire X Time, $F_{3,224} = 0.19$, $P = 0.903$) (Tab 1).

The evaluated morphological features such as leaf area and leaf length were significantly different between resprouting and non-resprouting plants. Concerning leaf

area, non-resprouting plants presented nearly half of leaf area of resprouting leaves, varied from 7.29 ± 0.47 cm² to 12.75 ± 0.60 cm², respectively (U = 74, N = 58, p = 0.00) (Fig. 2a).The leaf length varied between 4.37±0.16 and 5.81±0.15. Those were not disparate differences, but resprouting plants had a significantly higher mean leaf length (13.21±0.66 cm) than non-resprouting (7.32±0.48 cm) (t = -6.347, N = 58, p < 0.00) (Fig. 2b).

Herbivory by chewing insects and gall attacks

Non-resprouting plants were preferentially attacked by chewing insects – they showed almost the triple of leaf damage than resprouting plants (U = 604.5, N = 58, p = 0.004) (Fig. 3a). The proportion of leaves with damage by herbivores was also higher in non-resprouting plants than in resprouting ones, even though the actual removed leaf area by herbivores was similar in the different groups of plants (Tab. 1).

There was no difference in the leaf damage caused by *S. macrocapillata* and number of galls (larvae chamber) in non-resprouting and resprouting plants, even though non-resprouting plants had a little higher proportion of leaf damage by galls (Tab. 2). However, HR varied between 79.22% ± 5.53 (non-resprouting plants) and 91.97% ± 3.16 (resprouting plants) (t = -4.582, N=58, p = 0.000). The total of attack attempts was more than three times higher in resprouting than in non-resprouting plants (U = 144.5, N = 58, p = 0.000) (Fig. 3b), which shows a higher preference by *S. macrocapillata*. However, it also indicates a higher capacity of defense in those plants.

When we analyzed separately each group of plants and their physiological and morphological features, we observed that there was no correlation between non-resprouting features and herbivory rate or *S. macrocapillata* attacks (Tab. 3). Nevertheless, within resprounting plants there was a positive correlation between leaf

area and the index SPAD of chlorophyll (Spearman rho = 0.449, N = 58, p = 0.008) and leaf area and attempt of gall attacks (Spearman rho = 0.403, p = 0.038), which suggests that investments in growth and photosynthetic capacity in this group of plants. Thus, the capacity of nitrogen allocation to production of chlorophyll and the availability of resource in the leaf area are likely to be attractive to *S. macrocapillata* in resprouting plants.

Hypersensitive response and fire factor

The number of galls in plants varied from 4.680 ± 1.070 in 2014 to 6.360 ± 2.680 in 2015; the number of attack attempts related to gall incidence varied from 48.960 ± 6.987 to 68.600 ± 10.955. However, there was an increase of *B. brevipes* capacity to develop hypersensitive responses after fire; HR marks varied between 5.08 ± 0.98 in 2014 and 62.2 ± 10.6 in 2015 after fire (Wilcoxon test of plant resistance rate: W=324, N= 25, P = 0.000, Median = 52.50). An overall increase of 77% on *B. brevipes* resistance against *S. macrocapillata* was noticed, what 13 individuals whit 10% (or less) of resistance began to show more than 50% (Fig. 4).

Silicon content analysis

In general, the silicon rate varied between the individuals analyzed in this study. Resprouting plants show higher silicon contents than non-resprouting plants, with a mean of 0.15 ± 0.005% in dry biomass (Mann-Whitney Test: U = 233.50, N = 58, P = 0.004). However, there was no relation between silicon content and attempt of attacks by *S. macrocapillata* (ANCOVA of gall-midge incidence: Fire, $F_{1, 54}$. = 0.08, P = 0.784; Silicon, $F_{1, 54}$. =1.26, P= 0.267; Silicon *X* Fire, $F_{1, 54}$. = 0.92, P = 0.341) regarding fire, silicon. There was had no interaction between fire and silicon (Tab.4). The levels of leaf

damage by chewing-leaf herbivory varied negatively to silicon content (ANCOVA of herbivory rate: Fire, $F_{1, 54}$. = 0.79, P = 0.379; Silicon, $F_{1, 54}$. =10.44, P = 0.002; Silicon X Fire, $F_{1, 54}$. = 1.08, P = 0.304). Finally, herbivory rate decreases when silicon content increases in the leaves (Fig. 5) but this relation is no evidenced when the groups of plants are considered.

6.5 Discussion

Resprouting plants tend to show larger area and length leaf and higher chlorophyll concentration. It indicates more investment in growth and suggests more photosynthetic capacity and allocation of nitrogen to leaves. Changes in this set of features after fire can be seen in previous studies that record increase in the availability of resources (biomass accumulation), growth and photosynthesis rate (Wan et al. 2014) and enhance on plant nutritional quality (Vieira et al. 1996, Kay et al. 2007). These, reinforce the idea that resprouting plants are more attractive to a range of herbivore insects (Lopes & Vasconcelos 2011). However, other studies address decreases in nutritional quality of resprouting plants by physical defenses and accumulation of secondary compounds (Wheeler & Ordung 2006) or by the low digestibility of biomass (Schindler et al. 2014).

Resprouting plants of *Bauhinia brevipes* has lower herbivory rates by chewing insects than non-resprouting, even with greater vigor (see by the larger leaf area and length). Thus, our results go in an opposite direction if compared to some previous studies that report increases in herbivory rates among plants of burned areas (Stein et al. 1992, Kay et al. 2007, Lopes & Vasconcelos 2011). It is well known that the fire effects on physiologic plant features may indicate increase on chemical defenses such as terpenes (Lavoir et al. 2013), phenols (Wan et al. 2014), tannins (Shindler et al. 2004,

Wan et al. 2014) and resins (Hood et al 2015). This makes us believe that resprouting of in *B. brevipes* can be better defended than non-resprouting plants, once those can allocate resource, as nitrogen and others essential nutrients, to growth and defense.

While chewing insects showed poor performance in resprouting plants, *S. macrocapillata* showed preference for these plants by the higher rate of attacks in that plant group. For instance, these findings are consistent with the Plant Vigor Hypothesis (PVH), which predicts that the most vigorous plant or modules are preferably chosen by insects (Price 1991). Similarly to previous studies as Vieira et al. (1996) with gall-midges of *Contarinia* on *Palicourea rigida* and Lopes & Vasconcelos (2011) with galling herbivores on *Qualea grandiflora*, both studies relating the relation between gall attacks to plant vigor in burned plants of Brazilian cerrado.

Moreover, contrasting to the higher rate of attacks by *S. macrocapillata* in resprouting, we notice the high rate of induced defenses as hypersensitive responses within this plant group. This is confirmed by the rates of attack and defense in the same set of plants before (2014) and after (2015) the fire event. In these resprouting plants the attack rates (and number of galls) were similar throughout the years. However, we observed 77% overall increase on capacity of develop HR after fire (considering that several plants had no resistance before fire and began to show more than 50% after fire). This finds indicates that possible changes in the homeostasis of resprouting plants may be contributing to increase the tolerance and resistance to specific herbivores. In resproutings of *Pinus ponderosa* (Hood et al 2015) and *Populus tremuloides* (Wan et al 2014) there was an increase on contents of resins and tannins reducing specialized herbivory after fire events.

Fire can also be related to high rate of leaf silicon in *B. brevipes* resprouting, which may justify the low rate of herbivory by chewing insects in this plant group. This

finds agree with previous reports that relate the role of silica as mechanical defenses mechanism against herbivory (Korndörfer & Del-Claro 2006, Massey et al. 2006, Dias et al. 2014). Leaf toughness appears to be strongly correlated to silica accumulation in plant tissues and cell wall and reduce the digestibility of leaves (Massey et al. 2006). In *B. brevipes*, the role of silica as a defensive mechanism is clearly related to herbivory by chewing insects probably because it affects their buccal apparatus (Massey et al. 2006). In addition to this feature, free-living insects in general are less specialized in exploitation of food resources than endophytic insects. Thus, it enables us to infer that the increase in the contents of silicon in resprouting has direct effects on the foraging behavior of these insects (Hahn & Orrock 2014), making them avoids these plants.

This study provides the first information about fire mediated interactions between specialized and generalists herbivore insects, plant induced defense as hypersensitive responses and increase in leaf silicon concentration. Besides, the silicon role against herbivory is very clear, and this study also provides additional information about possible changes in foraging behavior of these herbivores in function of this nutrient. However, we also highlight the need for different approaches in future studies, testing plants in several perspectives of fire. It is also important to understand the patterns of plants responses to fire as regrowth ability among different species and individuals of the same species for future interventions in management of prone-ecosystem fire as the Brazilian cerrado.

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6.7 Tables

Table 1: Repeated measures ANOVA of chlorophyll content between group of plants (resprouting and non-resprouting) of *Bauhinia brevipes*. (*) $P < 0.05$

Source	*Df*	MS	*F*	*P*
Group plant	1	317.981	11.55	0.001 *
Time	3	133.744	4.86	0.003 *
Group plant X Time	3	5.220	0.19	0.903
Error	224	27.538		

Table 2: Comparison between resprouting and non-resprouting individuals regard to proportion of damaged leaves (damage by chewers or gall/leaves), the average of actual area removed of leaves (cm²) and leaf removed (%) in *Bauhinia brevipes* (Fabaceae) (Mean ± SE, N=58). The use of the Student's t-test was based upon normality tests. (*) $P < 0.05$. HR: Hypersensitive response.

Leaf damage	Variables	Resprouting	N-Resprouting	Statistic test	*P*-value
Chewing-leaf	Herbivory rate	0.011 ± 0.003	0.038 ± 0.732	U = 604.5	0.004*
	Damaged leaves	0.000 ± 0.000	0.001±0.000	U = 604.5	0.004*
	Removed area	0.140±0.039	0.267±0.053	U = 522	0.107
Gall midge	Damaged leaves	1.431±0.460	2.517 ± 0.709	U = 483.5	0.266
	Number gall	5.88 ± 2.50	4.13 ± 1.63	U = 466.5	0.412
	HR number	60.14 ± 10	16.09 ± 2.20	*t* = -4.582	0.000*
	Total attacks	66.037 ±10.355	20.226 ±2.686	U = 144.5	0.000*

Table 3: Spearman rank correlations in plant vigor (leaf-area, length and chlorophyll means) and herbivore by chewing-leaf insects and incidence of gall (gall number, HR marks); Spearman rho / *P*-value; (*) $P < 0.05$. Non-R: non-resprouting; R: resprouting.

Plant features	Herbivory rate (%)		HR marks		Total attacks	
	Non-R	R	Non-R	R	Non-R	R
Chlorophyll	0.165	0.120	-0.048	0.256	0.111	0.266
	0.376	0.552	0.797	0.198	0.551	0.181
Leaf area	-0.186	0.144	0.034	0.328	0.149	0.403
	0.317	0.473	0.857	0.095	0.425	0.038*
Leaf length	-0.083	0.072	0.033	0.173	0.197	0.240
	0.657	0.721	0.860	0.338	0.289	0.228

Table 4: Silicon effects on herbivory rate and hypersensitive responses in resprouting and non-resprounting individuals of *Bauhinia brevipes* examined by ANCOVA; (*) $P < 0.05$.

	Source	*Df*	MS	*F*	*P*
Herbivory rate	Fire	1	7.4828	10.44	0.002 *
	Silicon	1	0.5632	0.79	0.379
	Fire X Silicon	1	0.7710	1.08	0.304
	Error	54	38.6869		
HR marks	Fire	1	2.205	1.65	0.204
	Silicon	1	2.353	1.76	0.190
	Fire X Silicon	1	1.319	0.99	0.324
	Error	54	7.1987		

6.8 Figures

Figure 1. Differences in chlorophyll contents between resprouting and non-resprouting groups of *Bauhinia brevipes* (Fabaceae). The values are the mean ± standard error and letters represents the difference in means by Tukey pairwise comparisons. T1, T2, T3, and T4 = measures performed monthly.

Figure 2. Comparisons of (a) leaf area and (b) leaf length between resprouting and non-resprouting individuals of *B. brevipes* (Mean ± Confidence interval).

Figure 3. Comparisons of (a) herbivory rate and (b) attempts of attacks by *Schizomyia macrocapillata* (Diptera: Cecidomyiidae) (number of galls + hypersensitive responses) between resprouting and non-resprouting individuals of *B. brevipes* (Mean ± Confidence interval).

Figure 4. Hypersensitive response to *S. macrocapillata* (Diptera: Cecidomyiidae) before and after fire on individuals of *B. brevipes.*

Figure 5: Relationship between leaf silicon (%) and herbivory rate (%) on resprouting and non-resprouting individuals of *B. brevipes*.

Figure 1

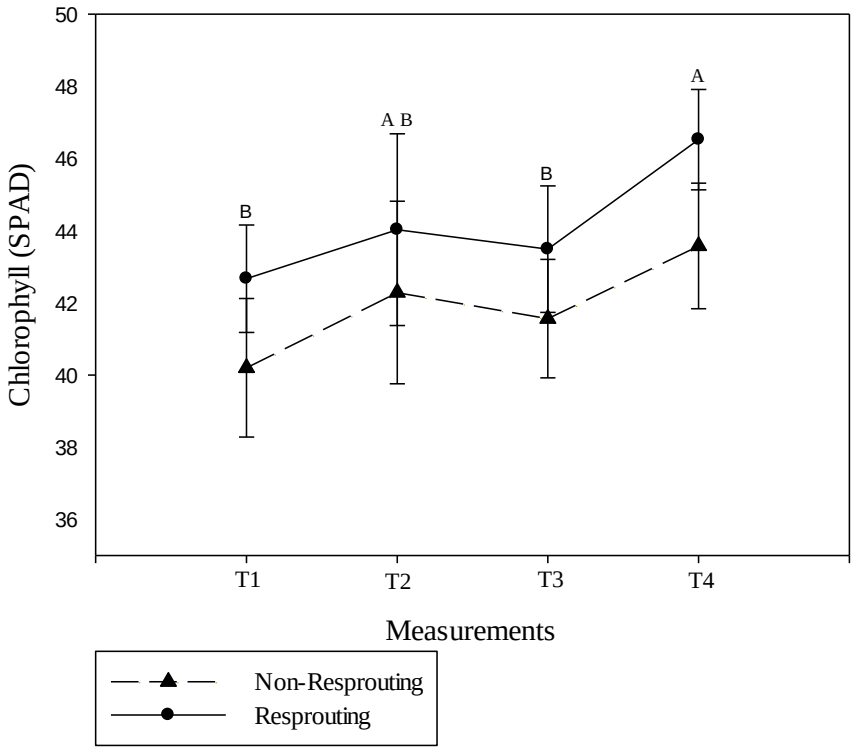

50
48
46
44
42
40
38
36
Chlorophyll (SPAD)
B
A B
B
A
T1
T2
T3
T4
Measurements
Non-Resprouting
Resprouting

Figure 2

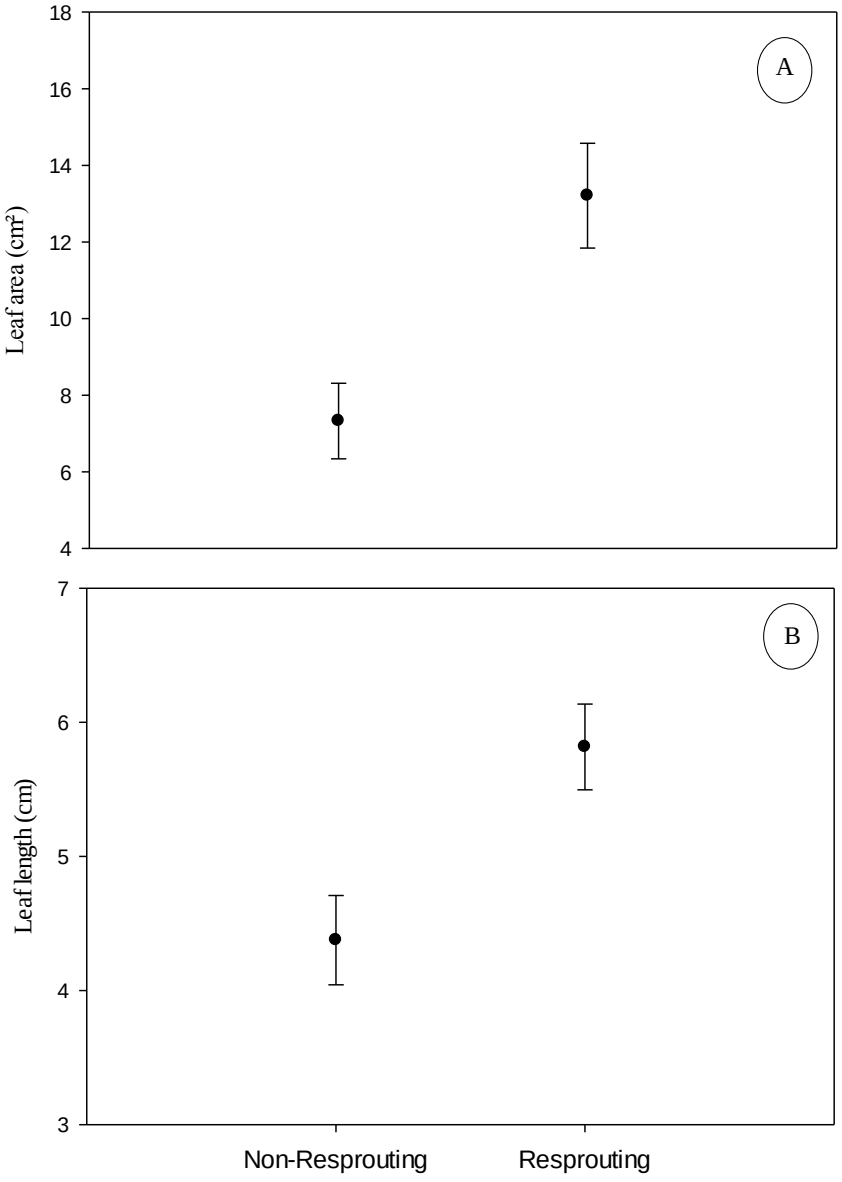

A
B
Leaf area (cm²)
Leaf length (cm)
18
16
14
12
10
8
6
4
7
6
5
4
3
Non-Resprouting
Resprouting
Group of plants

Figure 3

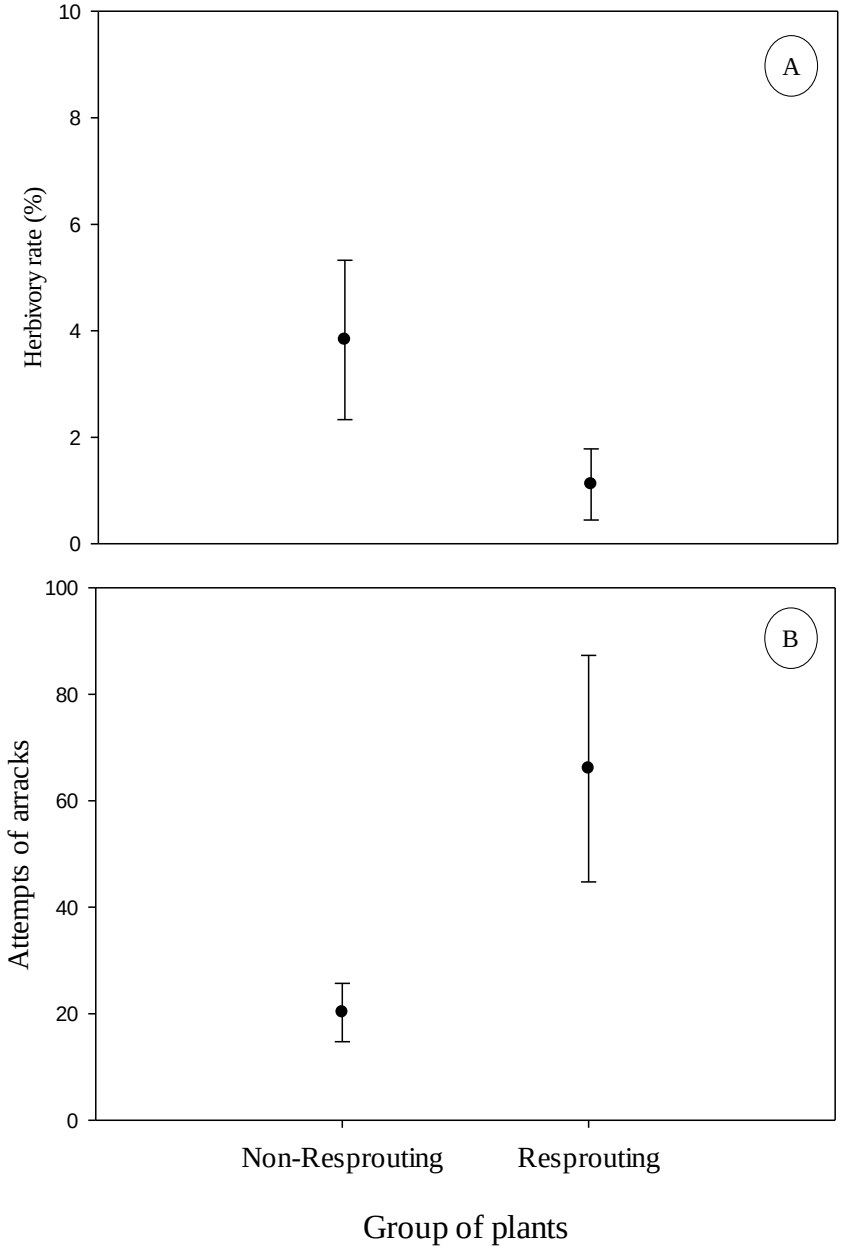

A
B
Herbivory rate (%)
Attempts of arracks
0
2
4
6
8
10
20
40
60
80
100
Non-Resprouting
Resprouting
Group of plants

Figure 4

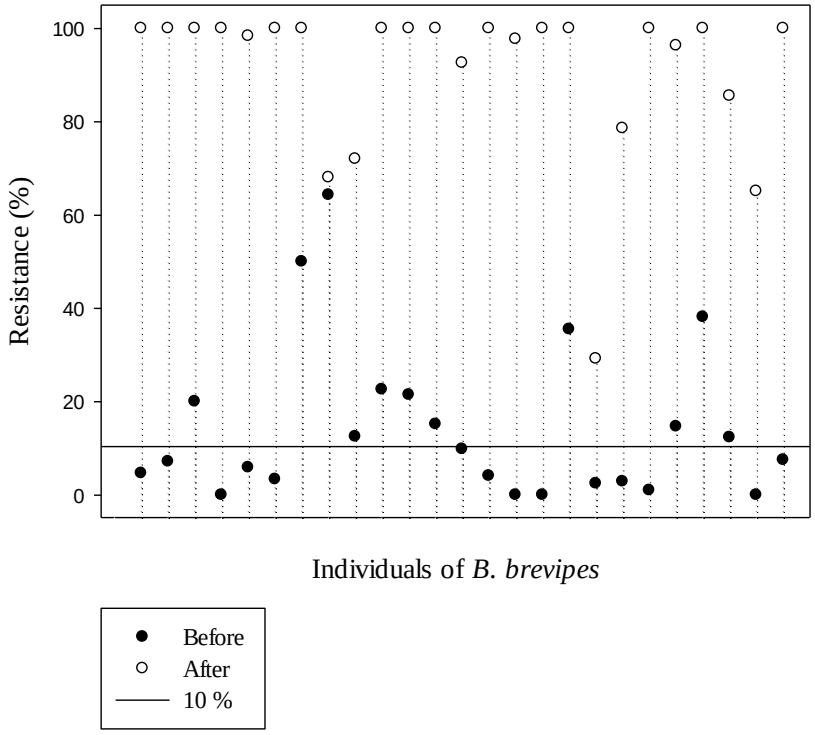
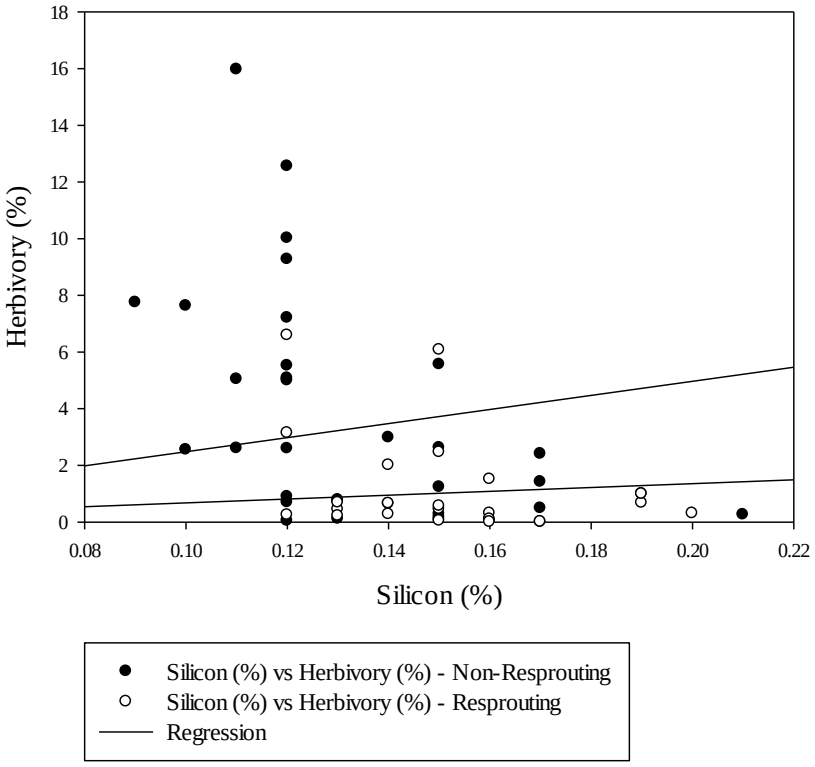

Resistance (%)
100
80
60
40
20
0
Individuals of *B. brevipes*
Before
After
10 %

Figure 5


Herbivory (%)
Silicon (%)
0.08
0.10
0.12
0.14
0.16
0.18
0.20
0.22
0
2
4
6
8
10
12
14
16
18
Silicon (%) vs Herbivory (%) - Non-Resprouting
Silicon (%) vs Herbivory (%) - Resprouting
Regression

7 CONSIDERAÇÕES FINAIS

A partir desse estudo, podemos verificar que *Bauhinia brevipes* possui uma marcada variação na capacidade de desenvolver defesas induzidas a partir do estímulo do galhador *Schizomyia macrocapillata*. Essa espécie também mantém associações com grupos de herbívoros de vida livre, principalmente insetos mastigadores. Além disso, observamos ainda marcado mecanismo de formação de novos ramos após eventos de fogo e a alocação de silício nas folhas.

No primeiro capítulo, evidenciamos claramente o desenvolvimento de respostas de hipersensibilidade em *B. brevipes* contra *S. macrocapillata*, confirmando o impedimento de grande parte das tentativas de ataque desse inseto galhador. No entanto, as plantas do fenótipo "resistente" de *B. brevipes* não mostrou valor adaptativo ao desempenho vegetativo (com relação ao crescimento e numero de folhas) e reprodutivo (produção geral de flores e relação com componentes do sucesso reprodutivo feminino e masculino). Igualmente, a prole proveniente desse fenótipo também apresentou similaridades no desempenho com a prole proveniente das plantas "susceptíveis", com investimentos em acumulo de reservas e crescimento bastante similares, independente do estágio de desenvolvimento analisado. Assim, esses resultados refutam a hipótese de que as plantas mais resistentes ao ataque de *S. macrocapillata* apresentam maior desempenho, uma vez que as plantas susceptíveis são similares às resistentes em termos de desempenho.

No segundo capítulo, verificamos que *B. brevipes* apresentou variações fisiológicas e morfológicas em resposta ao evento de fogo ocorrido em 2014, havendo expressiva emissão de novos ramos a partir do sistema subterrâneo de determinados indivíduos da população. Apesar desses indivíduos que rebrotaram apresentarem maior

área foliar, as taxas de herbivoria e ataque por *S. macrocapillata* foram menores quando comparadas aos indivíduos que não responderam ao fogo. Isso corrobora parcialmente a nossa hipótese que o fogo altera as interações entre insetos herbívoros e plantas hospedeiras. No entanto, se opõe à ideia que os indivíduos que rebrotaram são mais atrativos aos herbívoros, uma vez que esses também apresentaram maiores quantidades de respostas de hipersensibilidade e, consequentemente, menor numero de galhas. Verificamos também que a alocação de silício possivelmente reduziu o desempenho de insetos mastigadores, ou alterou comportamento de forrageamento dos mesmos, e também foi maior entre os indivíduos que rebrotaram. A partir desses resultados, acreditamos o fogo pode mediar as interações com insetos de diferentes guildas e em diferentes níveis, desde a formação de ramos mais vigorosos ao aumento no conteúdo de defesas mecânicas e induzidas.

Este estudo reforça a importância das respostas (ou reações) de hipersensibilidade como um eficiente mecanismo de defesas induzidas e da ação do fogo na mediação das interações. Contudo, sugerimos que estudos tratem de demandas conflitantes na alocação de recursos e crescimento e defesas induzidas, bem com defesas constitutivas. Acreditamos que o fogo tem importante papel na mediação das interações entre insetos e plantas hospedeiras através de respostas individuais de espécies adaptadas ao esse distúrbio como a alocação diferencial de nutrientes e o desenvolvimento de defesas induzidas, o que deve ser mais explorado em espécies do Cerrado.