Plant Ecol (2018) 219:69–78
https://doi.org/10.1007/s11258-017-0778-y

Effects of ferric soils on arthropod abundance and herbivory on *Tibouchina heteromalla* (Melastomataceae): is fluctuating asymmetry a good indicator of environmental stress?

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Received: 4 May 2017 / Accepted: 4 November 2017 / Published online: 9 November 2017
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Abstract High concentration of heavy metals in the soils represents an important factor of physiological stress that influences the normal functioning of plants through oxidation processes, and negatively affects insect performance and leaf consumption by herbivorous insects. One useful indicator to evaluate environmental stress in plants by heavy metals and herbivory is the fluctuating asymmetry, which describes the random differences in size or shape between the two sides of a bilateral character in organisms and it is a widely used measure of developmental instability in plants. We evaluated under natural conditions, the effects of variation of heavy metals in the soils on herbivore patterns, fluctuating asymmetry and arthropod abundance in *Tibouchina heteromalla* in rupestrian grasslands along the Espinhaço chain in Brazil. We selected two study areas, the first characterized by the presence of soils with low concentration of heavy metals (quartzite soils). In the second area, the soils are characterized by the presence of high concentration of heavy metals such as iron (ferric soils). We found that leaf thickness was higher in ferric soils than in quartzite soils. Conversely, total leaf area was greater in quartzite soils in comparison to ferric soils. Plants in soils with heavy metals had both lower herbivory levels and arthropod abundance than plants in soils with low concentrations of heavy metals. Fluctuating asymmetry levels were significantly greater in individuals from quartzite soils compared to individuals from ferric soils. Herbivory was positively related with individual fluctuating asymmetry in quartzite soils. Our results suggest that *T. heteromalla* presents tolerance to soils with heavy metals suggesting an acclimatization to these environmental conditions, and therefore, ferric soils may not represent a factor of environmental stress.

Communicated by Lori Biederman.

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Keywords Rupestrian grasslands · Plant stress · Differential herbivory · Herbivore guilds · Ironstone · Quartzite substrate

Introduction

Since plants are sessile organisms, they have evolved different physical (spines or trichomes) and chemical (phenols, tannins, alkaloids, and terpenes) defenses to protect against their natural enemies such as herbivorous insects (Coley and Barone 1996; Valkama et al. 2005). Chemical defense and plant nutritional quality affect the performance and host selection by insect herbivores (Agrawal and Fishbein 2006; Pascual-Alvarado et al. 2008; Johnson et al. 2014). Furthermore, abiotic factors such as temperature, humidity, and soil fertility influence the growth, performance, and distribution patterns of herbivore insects, resulting in changes in diversity and community structure of these insect guilds (Fernandes and Price 1988; Levesquea et al. 2002; Brudvig et al. 2015). Particularly, soil composition is one of the most important factors that indirectly affect insect community as result of changes in plant quality (Bertness and Hacker 1994; Cuevas-Reyes et al. 2004, 2011a). High concentration of certain heavy metals in soils, such as iron (Fe), and aluminum (Al) are retained in vegetative tissues (leaves and branches) and cause stress in plants, altering their normal function through oxidation process (Nagajyoti et al. 2010; Schaefer et al. 2016). Heavy metals produce significant morphological and biochemical responses of plants (Mangabeira et al. 2001; Preeti and Tripathi 2011). Specifically, there is a clear relation between heavy metal content in soil and morphological changes, such as alteration in cellular organization, leaf thickness, and reduced plant development (Zong et al. 2007; Yang et al. 2011). As a consequence, the structure and composition of the arthropod community can be modified through bottom-up effects (Poschenrieder et al. 2006; Noret et al. 2007; Ribeiro et al. 2016). The presence of heavy metals affects plant quality, which in turn, negatively influences insect performance, insect herbivory patterns, and their interactions with natural enemies and parasitoids. In this way, we might expect higher arthropod diversity in plants with lower concentrations of these metals (Clements 1988; Pollard and Baker 1997; Schmitz et al. 2000), and lower incidence and leaf consumption by herbivorous insects in plants with high concentration of heavy metals (Pollard and Baker 1997; Boyd 2004; Ribeiro et al. 2016).

One useful indicator to evaluate environmental stress in plants by both heavy metals and herbivory is the fluctuating asymmetry (FA) (Cuevas-Reyes et al. 2011b; Kozlov et al. 2015; Telhado et al. 2017). Fluctuating asymmetry describes morphological random deviations from perfect symmetry in bilateral structures of individuals (Møller 1997; Wilsey et al. 1998). Biotic and abiotic factors can increase FA levels causing alterations in developmental homeostasis at the chromosomal and epigenetic levels (Wilsey et al. 1998; Albarrán-Lara et al. 2010). For example, FA has been used to indicate the plant stress suffered by herbivory (Cornelissen and Stiling 2011; Cuevas-Reyes et al. 2011b; Fernandes et al. 2015), where FA increases with higher levels of herbivory (Alves-Silva and Del-Claro 2016). Similarly, some studies show that plants that occur in soils with high concentrations of heavy metals show also higher FA levels (Kozlov et al. 1996; Mal et al. 2002), but in others no relationship has been found (Ambo-Rappe et al. 2008).

To understand the ecology of plants that occur in this type of soil, it is necessary to analyze how the presence of heavy metals can influence both changes in plant quality, and herbivory patterns and arthropod abundance (Maldonado-Lopez et al. 2014). Some ecosystems present a primary edaphic composition of soils that contain naturally heavy metals, and represent low quality resources for many plant species (Nagajyoti et al. 2010; Rascio and Navari-Izzo 2011). These type of soils restrict the establishment of a great number of plant species and consequently may favor the presence of numerous endemisms (Fernandes 2016). This is the case of rupestrian grasslands, which have a mosaic of different soil types with low nutritional value and high concentration of heavy metals (Jacobi et al. 2007; Messias et al. 2013).

Tibouchina heteromalla (Melastomataceae) interacts with a wide variety of arthropods, including herbivores (Telhado et al. 2017). This plant species occurs in a mosaic of soils with high iron concentrations (ferric soils) and, in soils with low concentrations of this metal (quartzite soils), in rupestrian grasslands along the Espinhaço chain in Brazil. In this way, *T. heteromalla* represents an ideal model to evaluate the effects of different soils with variation in heavy metals on herbivore patterns, FA, and arthropod community. Our objectives were to (1) determine differences in herbivory levels and arthropod abundance in *T. heteromalla* populations, growing in quartzite and ferric soils; (2) analyze leaf morphological traits in plants growing in quartzite and ferric soils; (3) determine foliar fluctuating asymmetry levels in host plants that occur in quartzite and ferric soils; and (4) correlate the foliar fluctuating asymmetry levels with herbivory patterns and arthropod abundance.

Materials and methods

Study area

The study was conducted in two populations of *T. heteromalla* located in different rupestrian grassland regions in the Espinhaço mountain chain in southeastern Brazil. The rupestrian grassland ecosystem is characterized by the presence of sclerophyllous species and a rich endemism and biological diversity (Fernandes 2016). Two major rupestrian grasslands exist. The first site, characterized by the presence of soils with low concentration of heavy metals (quartzite soils) originated by the degradation of quartzite and arenitic rocks. In the second site, the soils present high concentration of heavy metals such as iron, aluminum, and manganese, originated from the decomposition of ferruginous substrates (ferric soils) (Furley and Ratter 1988; Benites et al. 2007; Schaefer et al. 2016). Both rupestrian grasslands originate a large mosaic of habitats. They are separated from each other, based on soil properties and water deposition. In both grasslands, stony and sandy grasslands, rock outcrops, and peat bogs are the most common habitats (Fernandes 2016). These soils are poor in nutrients with low availability of phosphorus and nitrogen (Oliveira et al. 2014).

The first study area was Serra do Cipó (19°10′–20′S, 43°30′–40′W), which is characterized by the presence of sclerophyllous shrubs and quartzite soils. The climate of this region presents dry winters and rainy summers, with an annual mean temperature of 21 °C and average annual precipitation of 1500 mm (Madeira and Fernandes 1999). The second study area was Serra da Moeda (20°11′–43′S, 43°58′–43′W), which is characterized by the presence of a surface composed by fragments of rocks distributed in a matrix of ferric soils (locally known as "Canga") (Carvalho Filho et al. 2010; Schaefer et al. 2016).

Study species

Tibouchina heteromalla Cogn. (Melastomataceae) occurs in Cerrado area, including regions of rupestrian grasslands (Guimarães and Martins 1997; Fernandes et al. 2015). It is a shrubby species of small size, which can reach up to 1.7 m. *T. heteromalla* has opposite fluted acorn-like leaves with smooth edges (Campos et al. 2009). This plant species maintains a high diversity of interactions with arthropods such as pollinators and herbivores (Fernandes et al. 2015).

Sampling design

We selected one population of *T. heteromalla* in each study area. In each area, we selected and marked 50 adult individuals with a similar height (Venâncio et al. 2016). To eliminate microhabitat effects, we standardized the sampling on each individual, randomly choosing three branches of the third order, with similar leaf size and quantity of leaves (Alves-Silva and Del-Claro 2013).

To evaluate the herbivory by insects, in each individual of *T. heteromalla,* we collected three leaves per branch and took a digital image of each leaf sampled and estimated the total leaf area and the area consumed by folivorous insects using the Image analysis software for plant disease quantification (Assess Image) (Cuevas-Reyes et al. 2011a). In addition, from the same individuals used for herbivory analysis, we randomly collected 10 leaves to determine the differences in leaf thickness using a Mitutoyo calliper (0.01 mm).

Arthropod census was conducted at the end of the rainy season. In each individual marked, we determined arthropod abundance using the beating technique (Neves et al. 2010). The arthropods collected were stored in 70% alcohol and identified at the lowest possible taxonomic level. Herbivores are kept at the insect collection of the Universidade Federal de Minas Gerais.

Fluctuating asymmetry

Fluctuating asymmetry was calculated in approximately 50 fully expanded intact mature leaves of each individual sampled in the two study areas. A digital image was taken for each leaf. We first standardized the measurements of leaf FA, considering the wider region of the leaves, which corresponds to the center of the leaf blade (Fernandes et al. 2015). Then, we made measurements of the right (*Ri*) and left side (*Li*) from the midrib to the edge of each side and estimated the absolute value of the difference between the distances from both sides ($|Ri - Li|$) divided by the average distance ($Ri + Li/2$) (Fig. 1) (Cuevas-Reyes et al. 2011b). To eliminate measurement error in FA, we selected a subsample of 100 leaves in each population studied. These leaves were re-measured without reference to previous measurements and then, we used a two-way ANOVA test to determine the significance of FA relative to measurement error (Cuevas-Reyes et al. 2011b). Finally, to verify of dependence between FA and another leaf trait, we used a linear regression analysis to determine the relationships between the absolute differences of leaf sides ($[Ri–Li]$) as a function of the width of each leaf (Palmer 1994) (quartzite soils: $F_{1,1010} = 85.8$; $R^2 = 0.78$; $P < 0.001$); (ferric soils: $F_{1,1075} = 95.9$; $R^2 = 0.8$; $P < 0.001$).

Statistical analysis

We performed a *t* test to determine the effects of type of soils (quartzite soils vs. ferric soils) on leaf thickness, total leaf area and herbivory, separately (Stokes et al. 2000). The model use type of soil as independent variable. Leaf area consumed by herbivore insects was used as response variable (SAS 2000). We performed a general lineal model (GLM) to identify the differences in arthropod abundance between host plants that occurs in quartzite soils and ferric soils. A Poisson error distribution and log link function were considered in the model.

A similar analysis was applied (GLM) to assess the effects of type of soil (quartzite soils vs. ferric soils) and herbivory, on foliar FA levels. Finally, in each study area, we used a linear regression analysis to determine the relationships between herbivory, FA, leaf thickness, and total leaf area (SAS 2000).

Results

Leaf thickness and total leaf area of *T. heteromalla* show difference between types of soil. We found that leaf thickness was higher in plants of habitats with ferric soils than in plants of quartzite soils ($t = 14.8_{1,999}$; $P < 0.0001$). In contrast, total leaf area was greater in plants of quartzite soils in comparison with plants of ferric soils ($t = 17.6_{1,999}$; $P < 0.0001$). The mean leaf thickness of plants growing in ferric soils was 0.06 ± 0.006 cm, while mean leaf thickness of plants in quartzite soils was 0.03 ± 0.009 cm (Fig. 2a). Plants growing on quartzite soils show a

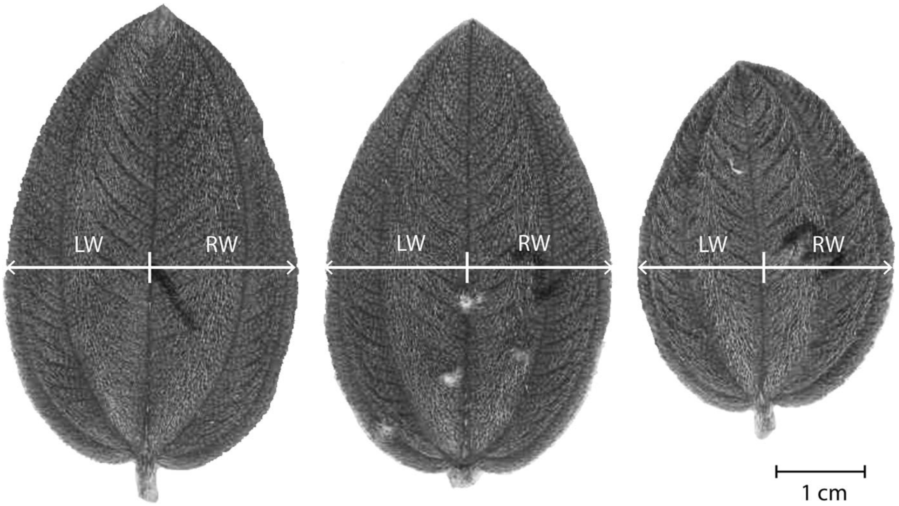

Fig. 1 Representation of the measurements used to estimate foliar FA in *Tibouchina heteromalla* (RW right width, LW left width) in leaves with different values of fluctuating asymmetry

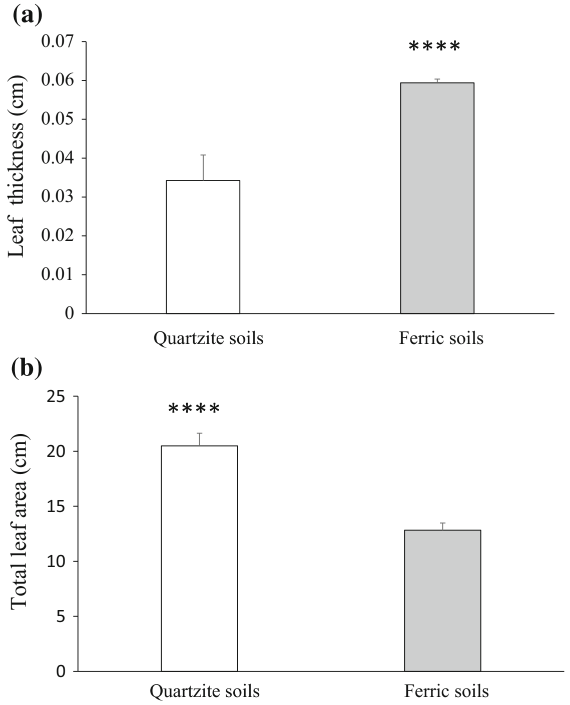

Fig. 2 Differences in host plant traits in individuals of *Tibouchina heteromalla* that occurs in quartzite soils and ferric soils in Rupestrian grasslands of Brazil. Leaf thickness (**a**). Total leaf area (**b**). The number of asterisks indicates the statistical significance of the observed differences (****$P < 0.0001$)

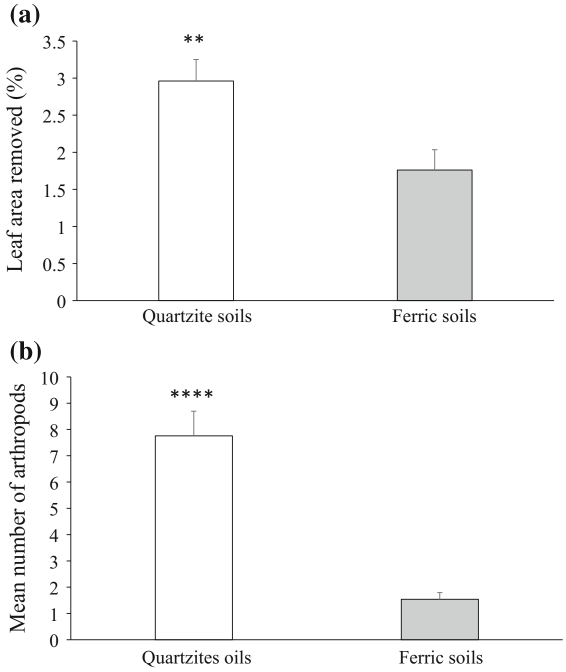

Fig. 3 Effects of type of soils on arthropods associated to *T. heteromalla*. Leaf area consumed by herbivore insects (**a**). Mean number of arthropods per host plant (**b**). The number of asterisks indicates the statistical significance of the observed differences (** $P < 0.001$; *** $P < 0.0001$)

mean of total leaf area of 20.5 ± 1.2 cm^2, while plants in ferric soils were 12.8 ± 0.65 cm^2 (Fig. 2b).

Leaf area consumed by herbivorous insects was higher in plants that occurred in quartzite soils than plants present in ferric soils ($t = 12.5_{1,299}$; $P < 0.007$). The mean leaf area consumed by herbivores in plants of quartzite soils was 3.0% ± 0.29, while the leaf area consumed on plants of ferric soils was 1.7% ± 0.27 (Fig. 3a). We found a positive relationship between leaf area consumed by herbivores and total leaf area in plants growing in quartzite soils ($F = 43.9$; $R^2 = 0.45$; $P < 0.001$). A negative relationship was found between leaf area consumed and leaf thickness in quartzite soils ($F = 66.9$; $R^2 = 0.68$; $P < 0.0001$) and in ferric soils ($F = 44.8$; $R^2 = 0.45$; $P < 0.001$).

We collected a total of 475 arthropods in both study sites (quartzite soil: 389; ferric soil: 86). The arthropods belong to eight orders. The two most abundant groups in both soil types were Hymenoptera (quartzite soil: 71%; ferric soil: 48%) and Hemiptera (quartzite soil: 26%; ferric soil: 27%). All collected phytophagous insects belonged to Aphididae family. In addition, we found that plants on quartzite soils present a higher mean number of arthropods per host plant in quartzite soils than in plants in ferric soils ($\chi^2 = 22.5$; d.f. = 1; $P < 0.0001$) (Fig. 3b).

FA values were significantly greater on leaves of individuals that occurred on quartzite soils than on plants of ferric soils ($\chi^2 = 17.5$; d.f. = 1; $P < 0.0001$) (Fig. 4). In addition, we found that FA was positively related with leaf area consumed by herbivores ($F = 69.1$; $R^2 = 0.64$; $P < 0.0001$) and with total leaf area ($F = 116.3$; $R^2 = 0.7$; $P < 0.0001$) in habitats of quartzite soils. Similarly, a positive relationship was found between total leaf area and leaf thickness ($F = 18.6$; $R^2 = 0.27$; $P < 0.001$). No relationship was found between FA and leaf thickness in plants growing in quartzite soils ($F = 0.33$; $R^2 = 0.004$; $P > 0.05$). Finally, we did not find significant relationship between FA and leaf area consumed by herbivores ($F = 0.13$; $R^2 = 0.002$; $P > 0.05$), leaf thickness ($F = 0.08$; $R^2 = 0.001$;

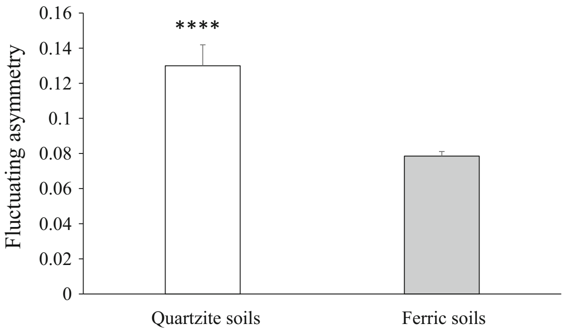

Fig. 4 Variation in foliar FA of *T. heteromalla* in quartzite soils and ferric soils in Rupestrian grasslands of Brazil. The number of asterisks indicates the statistical significance of the observed differences (*** $P < 0.0001$)

$P > 0.05$), and total leaf area ($F = 0.04$; $R^2 = 0.0009$; $P > 0.05$), respectively, in plants growing on ferric soils.

Discussion

Heavy metals are present in several types of soils as a result of both natural and anthropogenic activities (Wilson and Pyatt 2007). Some heavy metals such as chrome and cadmium are toxic to many plant species, while others like iron, zinc, and manganese are essential at low concentration for different physiological processes in a great number of plants (Costa 2000). When their concentration is high at supra-optimal values (i.e., above the redox potential range of aerobic cells stretches) (Schützendübel and Polle 2002), these essential heavy metals can be toxic, altering cellular and molecular processes by blocking both enzymatic activity and metabolic functional groups, affecting structural and anatomical plant traits, as a result of their accumulation in leaves and stems (La Rocca et al. 2009; Rascio and Navari-Izzo 2011). In the case of metallophytes plants (growing on naturally metal enriched soils), they show tolerance mechanisms to control uptake and avoid accumulation of heavy metals (Chardonnens et al. 1999). Many of these heavy metals enter in the plant and are maintained in the root cells, where a process of detoxification occurs when heavy metals are chelated by amino acids and organic acids or are compartmentalized in vacuoles (Chardonnens et al. 1999; Hall 2002). However, when the plant reaches the limit in storage capacity in specialized compartments, the whole plant needs to be involved. In this way, considerable proportion of these heavy metals are redirected into the epidermis, trichomes, and cuticle (Freeman et al. 2006a; Rascio and Navari-Izzo 2011). As a consequence, metallophytes plants, as *T. heteromalla,* show morphological changes with abnormal appearance, in comparison with individuals of the same species growing in soils with low concentrations of heavy metals. For example, there is a reduction in plant size, an increase in xeromorphic foliage characterized by thicker tissues with stenophylly (Wierzbicka and Rostanski 2002; Wójcik et al. 2017) and smaller and narrower leaves (Abratowska et al. 2012; Wójcik et al. 2013, 2017).

Our results corroborate these findings. Leaf thickness was higher and leaf area smaller in *T. heteromalla* individuals growing in ferric soils compared to plant individuals growing in quartzite soils. Endemic plants to areas of natural mineralization, such as *T. heteromalla,* can accumulate over 1% of heavy metals in their aerial organs resulting in the development of xeromorphic leaves that enable them to survive and to reproduce in this type of soils (Dahmani-Muller et al. 2000; Pulford and Watson 2003). As a consequence, leaf thickness and the incorporation of granular minerals into plant tissues play a very important role as structural defenses against herbivorous insects (Boege and Marquis 2005; Hanley et al. 2007; Ribeiro et al. 2016). In our case, plants with thinner leaves (i.e., from quartzite soils) had higher herbivory than plants with thicker leaves (i.e., from ferric soils). Therefore, the presence of heavy metals in soils could affect directly the structure and anatomy of leaves, and indirectly influenced the antagonistic interactions with the herbivores associated to *T. heteromalla.* Our results provide support for the “elemental defense” hypothesis (Martens and Boyd 1994) that proposes that high concentration of heavy metals in the aerial tissues of plants is a defensive strategy against the attack by natural enemies such as pathogens and herbivores. Three mechanisms have been proposed to explain this hypothesis. The first hypothesis called “avoidance” where the herbivore insect selectively fed on low-heavy-metal tissues; the second hypothesis “dietary dilution” consisting of consumption reduction of heavy metals in tissues by feeding on a combination of high and low heavy metal leaf tissues (Boyd 2007), and finally, the “tolerance” hypothesis that allows to specialist herbivore insects to consume plant tissues with high content of heavy metals, result of physiological adaptations (Boyd 2009; Rascio and Navari-Izzo 2011). However, because these hypotheses have been tested mainly in controlled laboratory conditions, the relationship between the presence of heavy metal in leaves and the incidence of herbivores is controversial in ecological literature. In some cases, higher concentration of leaf heavy metals is negatively related with the levels of herbivory (Jiang et al. 2005; Jhee et al. 2006), while in others cases, no relationship has been found (Huitson and Macnair 2003; Noret et al. 2007). In addition, our results are according to the study of Noret et al. (2007), who performed one of the first studies under natural conditions, demonstrating that populations of *Thlaspi caerulescens* with high zinc content in their foliar tissues experienced less amount of leaf area consumed than unmetalliferous populations, corroborating the influence of heavy metals on the preference of herbivorous insects.

Our results support the hypothesis that arthropod abundance is higher on plants that occur in quartzite soils in comparison with plants of ferric soils. Some studies show that preference and distribution of herbivore insects can be affected by plant chemical quality, which in turn, may influence the abundance of their natural enemies (Freeman et al. 2006b; Goncalves et al. 2007). A possible explanation for our results is that plants of ferric soils accumulate heavy metal in their tissues directly affecting herbivore incidence and indirectly the presence of their natural enemies through bottom-up effects (Behmer et al. 2005; Ribeiro et al. 2016). For example, a meta-analysis of the effects of heavy metals in the soil on plant–insect interactions (Butler and Trumble 2008) showed that herbivorous insects that feed on plants growing on metalliferous soils have a reduction in weight, growth, survival, fecundity, affecting the structure and composition of the food webs.

Contrary to expectations, FA levels were significantly greater in individuals from quartzite soils compared to individuals from ferric soils. Plants from quartzite soils present higher levels of herbivory that is positively related with FA levels. Considering that herbivory negatively affects growth and fitness of plants, these results suggest that herbivory directly may cause plant stress resulting in higher FA level. This agrees with other studies that have reported similar relationships between herbivory and FA levels, showing that the herbivory is an important factor that causes both, stress on their host plants and instability during their development (Møller and Shykoff 1999; Cuevas-Reyes et al. 2011b; Fernandes et al. 2015). Our results support the hypothesis that *T. heteromalla* is adapted to soils with heavy metals, and therefore, ferric soils may not represent a factor of environmental stress (Pulford and Watson 2003). In conclusion, our results show different relationships between heavy metals in soils of rupestrian grasslands and plant traits, and the consequences on plant–herbivore interactions on *T. heteromalla*. Hence, the study highlights the influence of heavy metals in the soil such as iron on the herbivory levels, and the importance of herbivorous insects as a factor of plant stressors which influence the FA levels.

Acknowledgements Pablo Cuevas-Reyes thanks Coordinación de la Investigación Científica, UMSNH for their generous support; GWF thanks the support provided by CNPq, Fapemig and Reserva Vellozia.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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