

# Seasonal variation of phenolic content in galled and non-galled tissues of *Calliandra brevipes* Benth (Fabaceae: Mimosoidae)

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## RESUMO

(Variação sazonal do conteúdo fenólico em tecidos galhados e não-galhados de *Calliandra brevipes* Benth (Fabaceae: Mimosoidae). Duas espécies, *Tanaostigmodes ringueleti* e *T. mecanga*, induzem galhas distintas em *Calliandra brevipes* Benth (Fabaceae: Mimosoidae), um morfotipo globoso e um fusiforme. Mudanças sazonais no conteúdo fenólico nos tecidos das duas galhas foram comparadas àquelas de folha e caule não galhados das plantas hospedeiras por um ano. A variação no perfil de conteúdo fenólico foi similar em tecidos galhados e não galhados, sendo associada primariamente às mudanças nos níveis de chuva, constituindo uma resposta direta ao estresse hídrico. Nos períodos de mudanças drásticas na precipitação de água, as alterações foram significativamente maiores em tecido não galhados do que em tecidos galhados, sugerindo que os galhadores estariam limitando a variação do conteúdo fenólico em seu próprio benefício.

**Palavras-chave:** *Tanaostigmodes ringueleti*, *Tanaostigmodes mecanga*, galha, interação inseto-plantas, fenol

## ABSTRACT

(Seasonal variation of phenolic content in galls and non-galled tissues of *Calliandra brevipes* Benth (Fabaceae: Mimosoidae)). Two species, *Tanaostigmodes ringueleti* and *T. mecanga*, induce distinct galls on *Calliandra brevipes* Benth (Fabaceae: Mimosoidae), a globose and a fusiform gall morphotype. Seasonal changes of phenolic content in the tissues of the two distinct galls were compared to those of non-galled leaves and stems of the host plants over one year. The variation in the phenolic content profiles was similar in both non-galled and galled tissues, and was primarily associated with changes in the levels of rainfall, indicating a direct response to hydric stress. In periods of drastic changes in water precipitation, the alterations were significantly higher in non-galled than in galled tissues suggesting that the gall inducers might limit the variation in the phenolic concentration for their own benefit.

**Key words:** *Tanaostigmodes ringueleti*, *Tanaostigmodes mecanga*, gall, insect-plant interaction, phenol

## Introduction

Gall formation represents the most intimate and specialized form of plant-herbivore interaction, and causes anatomical and metabolic alterations in the host plant functions, which provides shelter and food for insects or their offspring (Edwards & Wratten 1980; Fernandes & Price 1988; Hartley 1998; Schönrogge *et al.* 2000; Moura *et al.* 2008; Formiga *et al.* 2009). The most complex entomogenous galls are those induced by Cynipid wasps (Hymenoptera) (Schönrogge *et al.* 2000), such as *Tanaostigmodes* spp. which have been recently identified

in two species of Fabaceae (Mimosoidae): *Calliandra dsysantha* Benth (Perioto & Lara, 2005) and *C. brevipes* Benth (Penteado-Dias & Carvalho 2008). In these plant species, two new species of *Tanaostigmodes* (Hymenoptera: Tanaostigmatidae), *T. ringueleti* and *T. mecanga*, were identified in globose and fusiform galls, respectively (Carvalho *et al.* 2005; Penteado-Dias & Carvalho 2008). In a two-year effort to capture the gall inducers, *T. ringueleti* emerged only from the globose gall, which has a round and a larger axis, and is perpendicularly fixed to the stem. *Tanaostigmodes mecanga* appeared only in the fusiform gall, which is enlarged in its final third part, and has a perpendicular

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or oblique stalk coupled to the stem axis (Carvalho *et al.* 2005; Penteado-Dias & Carvalho 2008).

Previous histochemical studies found phenolic compounds in the cortex of both types of galls on *C. brevipes* (Carvalho *et al.* 2005). Until now, phenolic compounds in non-galled tissues have been associated to a defensive plant mechanism against the gall inducers, but these herbivores may also benefit from a secondary protection against the attack of their natural enemies (Abrahamson *et al.* 1991; Hartley 1998; Nyman & Julkunen-Titto 2000; Pascual-Alvarado *et al.* 2008; Formiga *et al.* 2009). Interpretations of analyses of compounds in gall systems generally relate them to a chemical defense against natural enemies, but few studies discuss the primary reasons for their synthesis as well as the control performed by gall inducers, especially in the Neotropical region.

Taking into consideration that abiotic factors may alter plant phenol content in the Neotropics (Formiga *et al.* 2009), it is of particular interest to study their influence on the phenolic content of *C. brevipes*. This specie is an interesting plant model that has two distinct morphological galls induced by two distinct insect species. Thus, to set future perspectives on the comparative studies of galls in the Neotropics, the present research compared seasonal variations of the phenolic content in non-galled tissues and in globose and fusiform galls.

## Materials and methods

The present study was performed at the *Campus* of the Universidade Federal de Juiz de Fora in Juiz de Fora (21°46'S and 43°21'W), Minas Gerais, Southeastern Brazil. The *Campus* is at an elevation of 678 a.s.l., and has a tropical climate, with an average annual temperature of 19.3 °C (Yacoub, 1998). Three groups of ten individuals of *Calliandra brevipes* were sampled. *Calliandra brevipes* Benth (Fabaceae: Mimosoidae) is a woody evergreen shrub native in South America formed by finely divided leaves and clusters of red soft and round surfaced flowers (Detoni *et al.* 2010). A voucher specimen (CESJ 31454) is deposited at the Leopoldo Krieger Herbarium of the Universidade Federal de Juiz de Fora, Minas Gerais, Brazil. The samples were collected bimonthly from August 2003 to August 2004 (one year). Non-galled tissues, named in this work as normal stem (NS) and normal leaf (NL), and the globose (GG) and the fusiform (FG) galls, both coupled to the stem, were removed from the host plants and washed with deionized water. The mature galls were dissected and the larva and/or adult insect was carefully removed to avoid interference of insect metabolites in the colorimetric assays.

To determine the phenol content, each tissue sample was weighted and processed immediately after being collected. Phenolic compounds from fresh NS, NL, GG and FG samples (1 g) were extracted during 1 h at 60° C, using 20 ml of ethanol/water (1:1). Total phenolic content was measured by the Folin-Dennis method (Waterman &

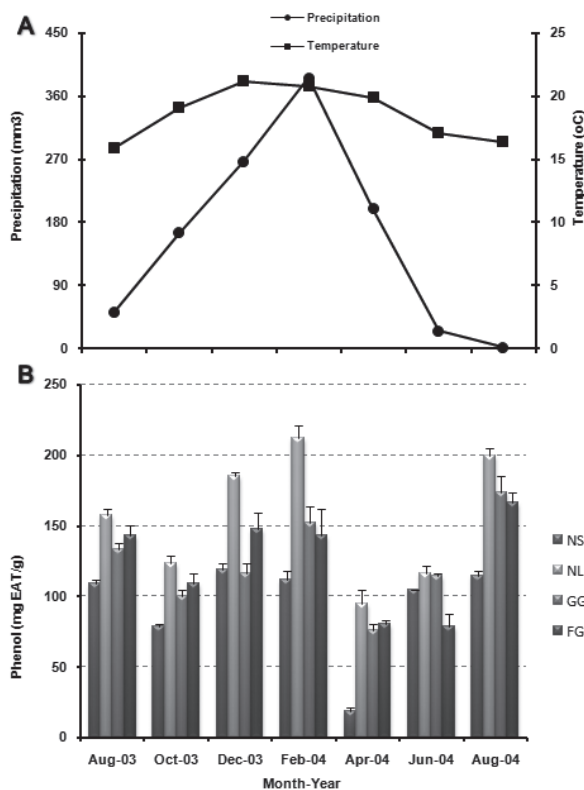
Mole 1994) using tannic acid as a standard, and expressed in mg/g of fresh plant tissue, in Tannic Acid Equivalents (TAE). The phenol data were obtained from five different extract samples, measured in triplicates, and analyzed using the ANOVA Tukey test (Graph Prism Software) to determine significant differences among the groups. P values < 0.05 were considered significant. A correlation analysis was done using the Graph Prism Software to evaluate if the phenol content was associated to abiotic factors.

## Results and discussion

The temperature and water precipitation data were collected from the Laboratório de Climatologia e Análise Ambiental of the Universidade Federal de Juiz de Fora, Minas Gerais. During the period of analysis, the minimum temperature (15.9° C) was registered in August 2003, which increased until December 2003, when the maximum temperature was registered (21.2° C; Fig. 1A). After this period, the temperature decreased progressively reaching 16.4° C in August 2004. The water precipitation increased from August 2003 to February 2004, followed by a slight increase in the temperature (Fig. 1A). After this period, water precipitation reduced progressively, and in August 2004, the lowest level was registered (Fig. 1A). These data suggest that the studied area has a dry period with lower temperatures (April 2004 to August 2004) and a rainy period with warmer temperatures (October, 2003 to April 2004), a seasonal profile very similar to that observed by Formiga *et al.* (2009) who related abiotic factors and phenolic content in galled tissues.

By analyzing the phenol content of normal tissues, it was possible to observe that the phenolic levels of NL were significantly ( $p < 0.001$ ) higher than those of NS from August 2003 to April 2004, and only in June 2004, the phenolic levels were similar in both NL and NS (Fig. 1B). After this period, the levels of phenolics increased in NL and NS, and a significant ( $p < 0.001$ ) difference between them reoccurred in August 2004 (Fig. 1B). In gall tissues, both GG and FG (Fig. 1B) showed a similar variation in their phenolic profile during the analyzed period. From February 2004 to April 2004, these levels significantly ( $p < 0.001$ ) decreased in a similar pattern (Fig. 1B).

In February 2004, concomitantly to higher water precipitation, the phenolic level in NL ( $211.9 \pm 8.52$  mg TAE/g) was significantly higher ( $p < 0.001$ ) than that observed in NS ( $111.9 \pm 5.32$  mg TAE/g), whereas the phenolic levels were similar between GG ( $152.4 \pm 10.65$  mg TAE/g) and FG ( $142.9 \pm 17.04$  mg TAE/g) (Fig. 1B). In April 2004, the phenolic levels in NL ( $95.24 \pm 8.52$  mg TAE/g), NS ( $19.05 \pm 2.13$  mg TAE/g), GG ( $76.19 \pm 4.26$  mg TAE/g) and FG ( $80.95 \pm 2.13$  mg TAE/g) significantly ( $p < 0.001$ ) decreased when compared to those found in the respective tissues in February 2004. Therefore, the variation in phenolic profiles was similar in all the samples, non-galled and galled tissues, a fact that is probably related to the oscillation in pluviometric levels (Fig. 1).



**Figure 1.** Seasonal variation in temperature and precipitation (A) and comparison of the phenolic content in normal (stem and leaf) and galled tissues, by two new species of *Tanaostigmatodes* (Hymenoptera: Tanaostigmatidae), *T. ringueleti* and *T. mecanga* (B), of *Calliandra brevipes* Benth (Fabaceae: Mimosoidae) in Juiz de Fora-MG, Southeastern Brazil. The concentration of phenolic compounds was measured in the fresh normal stem (NS) and leaf (NL), and in globose (GG) and fusiform (FG) gall tissues, using the Folin-Dennis method and tannic acid as a standard. The results (mg TAE/g) represent averages of three different experiments in triplicates, and the Standard Deviation is shown. The significant differences described in the text were determined by the ANOVA Tukey test.

To investigate if the phenol content variation is associated with the variation of abiotic factors, the correlation coefficient among these variables was measured. The data show that, from October 2003 to April 2004, there was a strong positive correlation ( $r = 0.78$ ) between water precipitation and the phenol content of plant tissues. On the other hand, when the variation in temperature was analyzed, a positive but moderate correlation was found ( $r = 0.67$ ) in relation to the phenol content. These findings showed that, in the studied region, the water precipitation contributed more to the variation on the phenolic contents of *C. brevipes* than the oscillation in the temperature. After this period (see April to August, 2004), while the water precipitation continuously lowered (Fig. 1A; 199.7 to 2 mm), the phenolic levels in all tissue samples increased again, and reached their maximum in August 2004 (Fig. 1B,  $r = -0.79$ ).

The water deficit increases oxidative stress with an accumulation of active oxygen species and an alteration in antioxidant molecule content (Dat *et al.* 2000; Feng *et al.* 2008). Phenolic compounds contribute to absorbing and neutralizing free radicals, and it has been suggested that phenolics

found in galls may represent a defense mechanism against oxidative stress (Soares *et al.* 2000, Detoni *et al.* 2010). Therefore, the significant changes in phenolic content in both non-galled and galled tissues could be a primary response to changes in water precipitation in the studied region, which could be considered a mode of scavenging free radicals generated by hydric stress in plant tissues. Interestingly, although the variation in the phenolic profile in all tissues seemed similar in relation to the abiotic factors, in a more careful analysis, it could be noticed that the amplitude of these variations was comparatively more drastic in normal tissues than in galled ones during the whole period of analysis (October 2003 to August 2004) (Fig. 1, A and B). Therefore, the two distinct species of *Tanaostigmatodes* manipulated and limited the variation of total phenolic levels in their gall tissues in a similar way, probably for its own defense. In conclusion, our results suggested that water precipitation generates the primary stimuli for the accumulation of these compounds, motivating the analyses of other parameters and metabolites. Our results corroborate Formiga *et al.* (2009), showing that the abiotic factors and the galls act together modulating the host chemical response. The comparison of the concentrations of the metabolites in non-galled tissues and in distinct galls co-occurring in the same host plant may indicate the insect's ability to the manipulate plant metabolism as well as reveal plant constraints to this ability.

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