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NPK and flavonoids affecting insect populations in *Dimorphandra mollis* seedlings

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ABSTRACT. The study evaluated the influence of different levels of nitrogen (N), phosphorus (P) and potassium (K), and flavonoids on the population of insects in *Dimorphandra mollis* Benth (Leguminosae) seedlings. The treatments associated with the highest level of attacks by *Frankliniella schulzei* (Trybon) (Thysanoptera: Thripidae) were 600 mg dm^{-3} of P and 50 mg dm^{-3} of K. The highest level of attacks by Coccidae occurred for 300 of P and 150 and 250 mg dm^{-3} of K. The last two treatments also exhibited the highest level of attacks by Pseudococcidae. On the other hand, the control exhibited higher levels of flavonoids and a lower level of insect attacks. We observed a small positive effect of N levels on attack by *F. schulzei*. The levels of N, P and K negatively affected the levels of flavonoids in the leaves of *D. mollis*. We detected no significant effects of flavonoid levels on the populations of Coccidae, Pseudoccocidae and *F. schulzei*. Higher numbers of Coccidae and Pseudococcidae were observed in the abaxial face of apical leaves. However, higher numbers of *F. schulzei* were observed on the adaxial face at lower heights in the canopy. The preferred treatment for the production of *D. mollis* seedlings is the control (without fertilization) because it showed higher flavonoid levels than other treatments and did not result in higher insect numbers.

Keywords: “fava d’anta”, Coccidae, Pseudococcidae, *Frankliniella schulzei*.

NPK e flavonoides afetando populações de insetos em mudas de *Dimorphandra mollis*

RESUMO. O estudo avaliou a influência de diferentes níveis de nitrogênio (N), fósforo (P) e potássio (K) e flavonoides sobre a população de insetos em mudas de *Dimorphandra mollis* Benth (Leguminosae). Os tratamentos associados com maiores níveis de ataque de *Frankliniella schulzei* (Trybon) (Thysanoptera: Thripidae) foram 600 mg dm^{-3} de P e 50 mg dm^{-3} de K. Os maiores níveis de ataque de Coccidae ocorreu para 300 de P e 150 e 250 mg dm^{-3} de K. Os dois últimos tratamentos também exibiram altos níveis de ataque de Pseudococcidae. Por outro lado, o controle exibiu maior nível de flavonoides e menor nível de ataque de insetos. Foi observado um pequeno efeito positivo dos níveis de N no ataque de *F. schulzei*. Os níveis de N, P e K afetaram negativamente os níveis de flavonoides nas folhas de *D. mollis*. Não foi detectado efeitos significativos dos níveis de flavonoides sobre as populações de Coccidae, Pseudoccocidae e *F. schulzei*. Observou-se maior número de Coccidae e Pseudococcidae na face abaxial de folhas apicais. Entretanto, maiores números de *F. schulzei* foram observados na face adaxial das partes mais baixas do dossel. O tratamento preferido para produção de mudas de *D. mollis* é o controle (sem fertilização) porque apresentou maior teor de flavonoides do que nos outros tratamentos e não apresentou maior ataque de insetos.

Palavras-chave: fava-d’anta, Coccidae, Pseudococcidae, *Frankliniella schulzei*.

Introduction

The use of medicinal plants to cure or to prevent disease increases each year. The cultivation of these plants and/or the extraction of substances from plants that possess therapeutic properties has become an attractive alternative for agriculture and is interesting and viable at the national level of the industry (SIMÕES et al., 2000). However, some species that show great potential for the pharmaceutical industry are not receiving sufficient attention and are little studied by the scientific community.

Among the species of the Brazilian flora that deserve more extensive study is *Dimorphandra mollis* Benth (Leguminosae), commonly known as “fava d’anta”, a native species of the Brazilian Savanna, found in Minas Gerais, São Paulo, Goiás, Mato Grosso and Mato Grosso do Sul (LORENZI; MATOS, 2002; PACHECO et al., 2010). The *D. mollis* is a deciduous pioneer tree of medium size. The bioflavonoid rutin or rutoside is extracted from its fruits in the prematuration stage (LORENZI; MATOS, 2002; LUCCI; MAZZAFERA, 2009). The proportion of rutin occurring in the dry matter can vary from 6 to 10% (HUBINGER et al., 2010; SOUZA et al., 1991). Other substances, such as rhamnose and quercetin, can also be extracted from the fruits (PETACCI et al., 2010).

Species of plants such as *D. mollis* that are subject to intensive extraction activities are thereby at risk for extinction. Accordingly, such species require management strategies and cultivation procedures (GONÇALVES et al., 2010; LEITE et al., 2006; VIANA E SOUZA; LOVATO, 2010). An alternative is the production of seedlings for colonization of the areas in which extractive activity occurs or for commercial plantations. This approach requires knowledge of the nutritional demands of the plants, primarily in regard to such macronutrients as nitrogen (N), potassium (K) and phosphorus (P). Consequently, the domestication of the medicinal plants is an alternative approach for the production of Phytotherapeutic substances for the industry. However, the seedlings can be attacked by insects that can affect the quantity and the quality of the product (LEITE et al., 2008).

Moreover, the fertilization of plants can influence vulnerability to insect attack (LEITE et al., 2003). Indeed, different levels of nutrients can produce physiological and morphological alterations in plants (COELHO et al., 1999; LARA, 1991). Food has very significant effects on the distribution and abundance of insects. It has direct influences on insect populations and affects biological, morphological and behavioral processes (GALLO et al., 2002). The nutrients N, P and K have important functions in plants and interfere with insect population dynamics. Previous studies of insects associated with *D. mollis* have investigated ants and bees in adult plants (CINTRA et al., 2002, 2005a and b; ROTHER et al., 2009).

The aim of this work is to evaluate the influence of N, P and K levels and flavonoids on the insects associated with *D. mollis* seedlings.

Material and methods

These studies were conducted under greenhouse conditions in the "Instituto de Ciências Agrárias da Universidade Federal de Minas Gerais (ICA-UFMG)", Montes Claros, Minas Gerais State, from July to November 2005.

Seeds of *D. mollis* were subjected to scarification on the side opposite the hilum and were immersed in water for 24h. Soon thereafter, six seeds were planted in a three dm^3 vase. The soil used was a dystrophic Red Latosol collected from a layer 0-20 cm deep. Soil physical and chemical properties, determined according to the methodology of Embrapa (1997), were as follows: pH in water 4.6, P = 0.6 mg dm^{-3}, Ca = 11.0 $mmol_c$ dm^{-3}, Mg = 4.0 $mmol_c$ dm^{-3}, K = 1.0 $mmol_c$ dm^{-3}, Al = 37 $mmol_c$ dm^{-3}, H + Al = 140 $mmol_c$ dm^{-3}, S = 16.0 $mmol_c$ dm^{-3}, t = 53.0, m = 70%, T = 156 $mmol_c$ dm^{-3}, V = 10%, organic matter = 24 g kg^{-1}, sand = 500 g kg^{-1}, silt = 80 g kg^{-1} and clay = 420 g kg^{-1}.

After germination, the two largest plants were chosen from each pot and the other plants eliminated. The experimental design was entirely randomized with three repetitions and 19 treatments: six levels of nitrogen (50, 100, 150, 200, 250 and 300 mg dm^{-3}) in the form of NH_4NO_3, six levels of phosphorus (100, 200, 300, 400, 500, 600 mg dm^{-3}) in the form of H_3PO_4 six levels of potassium (50, 100, 150, 200, 250 and 300 mg dm^{-3}) in the form of KNO_3, and the control (without fertilization).

To complement the fertilization, the nutrients Mg, S, B, Cu, Zn and Ca were used (60, 40, 0.35, 1.5, 0.5, and 200 mg dm^{-3}, respectively). Fertilization was performed one week before the seeds were planted. Irrigation was conducted with distilled water in order to avoid contamination and to ensure the effects of the fertilization.

The aerial part of each plant was collected after 150 days, at the beginning of December 2005. The aerial part was placed in Kraft paper bags, dried in a forced-circulation oven at 60°C for three days until constant weight was achieved, and used for determination of dry matter. Subsequently, this material was ground in a Wiley mill (20 mesh), and the extraction of total flavonoids was performed according to Mendes et al. (2005).

The evaluations of insect occurrence (natural infestation) and of the level of damage (percent) were performed by visual inspection weekly in the morning on one leaf from the apical, medium and basal parts of the canopy of each plant, on both leaf faces. Defoliation was determined visually by estimating the percentage of leaf area lost on a scale from 0-100% by increments of 5% of the total area removed (SASTAWA et al., 2004, MIZUMACHI et al., 2006).

Regression analysis ($p < 0.05$) was applied to relate data on insects to N, P and K and flavonoids and to relate flavonoids to soil attributes. Data were transformed using the square root function $\sqrt{x+0.5}$ and subjected to an analysis of variance and to the Tukey or Scott-Knott tests ($p < 0.05$).

Results and discussion

Scale insects of the families Coccidae and Pseudococcidae (Hemiptera) and thrips *Frankliniella schulzei* (Trybon) (Thysanoptera: Thripidae) were observed in *D. mollis* seedlings. The attack of scale insects produced honeydew on the *D. mollis* leaves. These families of scale insects include the pest species *Coccus viridis* (Green) in citrus culture and *Pseudococcus adonidum* (L.) in horticultural plants. *Frankliniella schulzei* produced scratches on the leaves of *D. mollis* seedlings and thereby caused leaf chlorosis. This insect is a pest species on several crops including cotton and horticultural plants (GALLO et al., 2002).

The treatments associated with the highest level of attacks by *F. schulzei* were 600 mg dm^{-3} of P and 50 mg dm^{-3} of K (Table 1). The highest level of attacks by Coccidae occurred for 300 mg dm^{-3} of P and 150 and 250 mg dm^{-3} of K. The last two treatments also exhibited the highest level of attacks by Pseudococcidae (Table 1). The control exhibited higher levels of flavonoids and a lower level of insect attacks (Table 1).

Table 1. Effect of nitrogen (N), potassium (K) and phosphorus (P) on the leaf damage (%) produced by thrips *Frankliniella schulzei* and on the number of Pseudococcidae and Coccidae $leaf^{-1}$ face and flavonoid levels (% dry matter) in *Dimorphandra mollis* seedlings.

Treatments	Leaf damage (%)	Coccidae	Pseudo-coccidae	Flavonoids (%)
Without fertilization	0.43 b	0 b	0 b	4.59 a
50 mg dm^{-3} of N	0.92 b	0.01 b	0 b	1.78 b
100 mg dm^{-3} of N	1.03 b	0.03 b	0 b	1.88 b
150 mg dm^{-3} of N	1.47 b	0 b	0 b	1.74 b
200 mg dm^{-3} of N	0.79 b	0 b	0 b	1.64 b
250 mg dm^{-3} of N	1.36 b	0.01 b	0 b	1.53 b
300 mg dm^{-3} of N	0.33 b	0 b	0 b	1.83 b
100 mg dm^{-3} of P	0.86 b	0 b	0 b	1.67 b
200 mg dm^{-3} of P	1.00 b	0.09 b	0.010 b	1.40 b
300 mg dm^{-3} of P	0.94 b	0.22 a	0.006 b	1.83 b
400 mg dm^{-3} of P	1.49 b	0 b	0 b	1.19 b
500 mg dm^{-3} of P	1.17 b	0 b	0 b	1.56 b
600 mg dm^{-3} of P	3.06 a	0.05 b	0 b	1.39 b
50 mg dm^{-3} of K	2.73 a	0.12 b	0.006 b	1.98 b
100 mg dm^{-3} of K	1.09 b	0.07 b	0.006 b	2.04 b
150 mg dm^{-3} of K	1.16 b	0.30 a	0.078 a	1.60 b
200 mg dm^{-3} of K	1.31 b	0.02 b	0 b	1.75 b
250 mg dm^{-3} of K	1.26 b	0.21 a	0.073 a	1.99 b
300 mg dm^{-3} of K	1.08 b	0 b	0.006 b	1.78 b

The following averages for the same letter in the line do not differ for the Scott-Knott test to 5% of probability.

Excess of N or deficiency of K can cause accumulation of free amino acids and can consequently increase the populations of sucking insects on plants (JANSSON; EKBOM, 2002). Species of sucking insects such as aphids, scale, leafhoppers, whitefly and thrips and several species of phytophagous mites are not able to extract amino acids from proteins for their own use. Consequently, these insects depend on free amino acids in the plants (GALLO et al., 2002). Phosphorus can participate in cell wall synthesis. It can accordingly influence the function of the cell wall as a barrier to disease. Phosphorus can also occur in toxic compounds and can affect metabolic routes that counteract insects or diseases (MALAVOLTA, 2004). We noted a small positive effect of N levels on attack by *F. schulzei* (Figure 1). However, the effects of N, P and K on attacks by Coccidae and Pseudococcidae as well as the effects of P and K in relation to *F. schulzei* were not significant (data not shown). In other words, we did not observe a substantial effect of the levels of N, P and K on the insects. This finding perhaps results from the low population densities of insects on *D. mollis* seedlings (Table 1).

Dimorphandra mollis participates in a symbiosis with soil bacteria that assimilate atmospheric N (PEREIRA; OLIVEIRA, 2005). Probably for this reason, the N levels did not affect the growth of the seedlings. According to Pinto and Lameira (2001), the synthesis of compounds originating from secondary metabolism can be induced in several ways; for example, by nutritional stress. Mendes et al. (2005) also verified a reduction in the levels of total flavonoids as a function of increasing doses of P in *D. mollis* seedlings cultivated in nutrient-rich solution. In the present experiment, the stressed seedlings (without fertilization) produced larger quantities of secondary metabolites, including flavonoids (Figure 2). These seedlings were not preferred by the insects (Table 1). Gazzoni et al. (1997) observed higher mortality in caterpillars given a diet of mixed flavonoids (rutin and quercetin) at the highest doses.

We detected no significant effect of flavonoid levels on the scale insects (Coccidae + Pseudoccocidae) and *F. schulzei* populations (Figure 1). The flavonoids are probably important as an initial barrier against insects (antixenosis). Once the insects were established on the plants, they may have experienced deleterious effects on their life cycles (antibiosis). This process would explain their low density in *D. mollis* seedlings. In other words, the lowest level of flavonoids observed in *D. mollis* leaves (1.02%) could have been enough to affect these insects negatively. Macedo et al. (2002) observed a strong negative effect on *Callosobruchus maculates* F. (Coleoptera: Bruchidae) larvae of 1% trypsin inhibitor isolated from *D. mollis* seeds. According to Fernandes et al. (2004), an increase in the concentration of nitrogen in the plant reduces the concentration of substances related to defense.

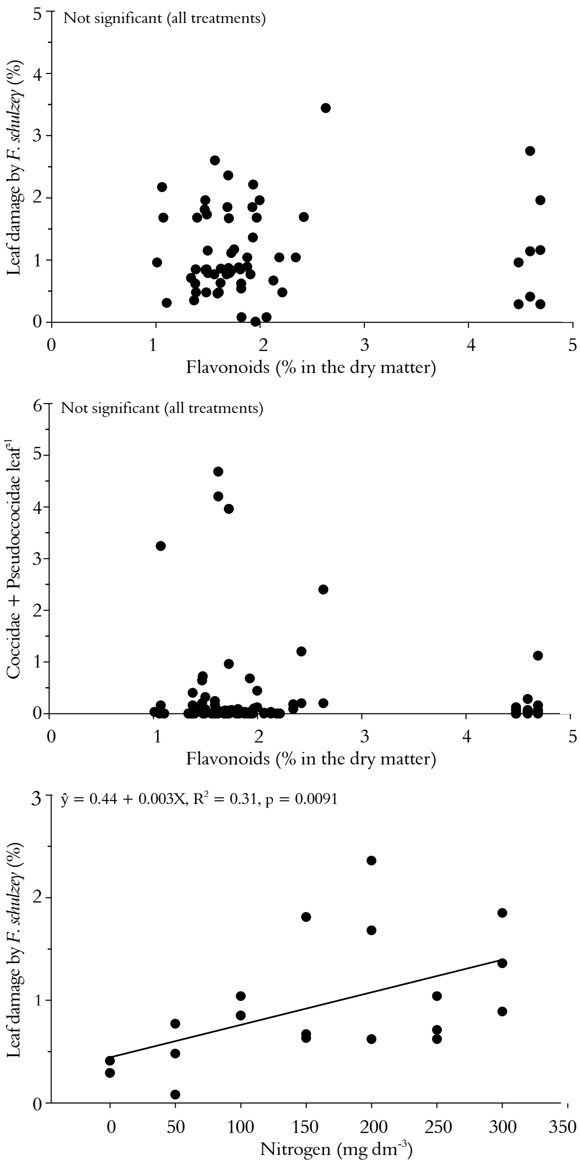

Figura 1. Effects of flavonoids levels on the attack of *Frankliniella schulzei* and Coccidae + Pseudococcidae populations, and nitrogen levels on the attack of *F. schulzein* in leaves of *Dimorphandra mollis* seedilings. Each symbol represent one repetition.

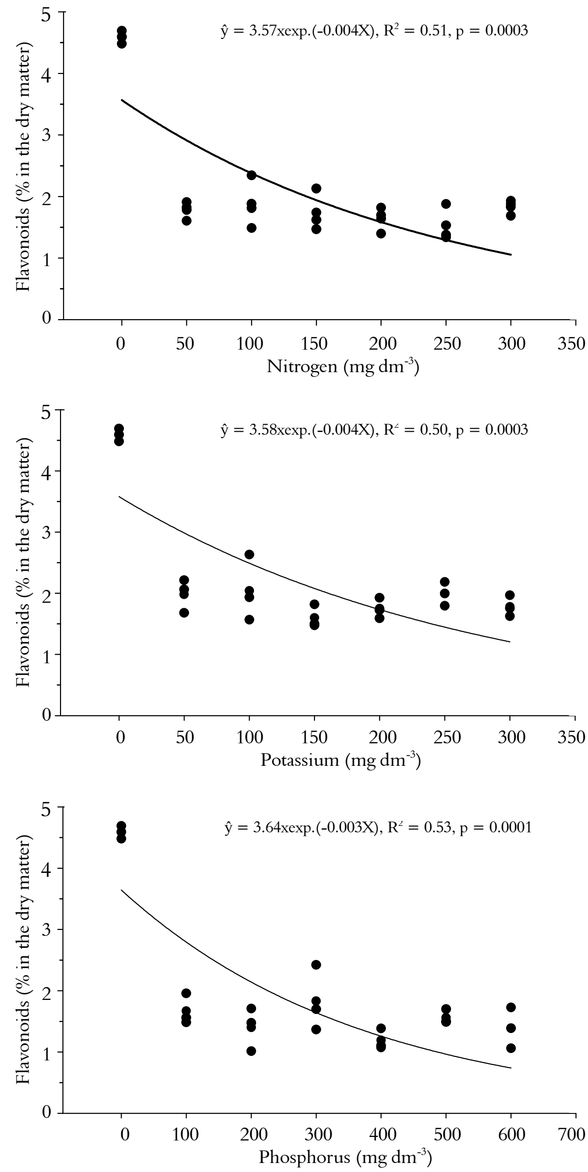

Figura 2. Effects of levels of nitrogen, potassium and phosphorus on the flavonoids levels in leaves of *Dimorphandra mollis* seedlings. Each symbol represent one repetition.

These substances include carbon-rich tannins and terpenes. The quantity and quality of the soluble compounds of nitrogen produced depend on the source of nitrogen used. These compounds could induce a larger or a smaller degree of pest resistance in the plant (BORTOLI; MAIA, 1994). Therefore, the effect of NPK on the insects is both direct (nutritional value of the plant) and indirect (defense of the plant), as observed in this work.

In all the treatments, the numbers of Coccidae and Pseudococcidae observed on the abaxial face on apical leaves were higher than the numbers observed on plants in the medium and basal heights of the canopy (Table 2). However, higher numbers of *F. schulzei* were observed on the adaxial face of the leaves of the inferior third of the canopy (Table 2). This distribution probably resulted from competition for space and food among species. Insects, particularly sucking insects, attack the abaxial face of apical leaves because these parts of the plant are more tender (the quantities of calcium and fiber are smaller) and are of higher nutritional value (higher level of N) (SILVA et al., 1998; LEITE et al., 2002; CHAU et al., 2005; SANTOS et al., 2003).

Table 2. Effect of canopy height and leaf face on leaf damage (%) and on the numbers of thrips *Frankliniella schulzei*, Pseudococcidae and Coccidae leaf^{-1} face in *Dimorphandra mollis* seedlings under different concentrations of nitrogen (N), phosphorus (P) and potassium (K) in the soil.

Insects	Canopy height of plants under different N levels		
	Apical	Medium	Bottom
Thrips damage (%)	0.00 b	0.24 b	3.87 a
Thrips	0.00 b	0.01 ab	0.02 a
Coccidae	0.033 a	0.012 ab	0.006 b
Pseudococcidae	0.027 a	0.004 b	0.001 b
	Plants under different P levels		
Thrips damage (%)	0.00 b	0.41 b	5.21 a
Pseudococcidae	0.017 a	0.010 ab	0.000 b
	Plants under different K levels		
Thrips damage (%)	0.00 b	0.31 b	5.06 a
Coccidae	0.30 a	0.17 ab	0.03 b
Pseudococcidae	0.11 a	0.10 ab	0.01 b

Insects	Leaf face of plants under different N levels	
	Adaxial	Abaxial
Thrips	0.02 a	0.01b
Coccidae	0.00 b	0.03 a
Pseudococcidae	0.00 b	0.02 a
	Plants under different P levels	
Coccidae	0.00 b	0.14 a
Pseudococcidae	0.00 b	0.02 a
	Plants under different K levels	
Thrips	0.01 a	0.00 b
Coccidae	0.00 b	0.33 a
Pseudococcidae	0.00 b	0.14 a

The following averages for the same letter in the line do not differ for the test of Tukey to 5% of probability.

Conclusion

Scale insects can be pests of *D. mollis* seedlings because they suck the sap and coat the leaf with honeydew, thereby facilitating the growth of soot mold. Thrips can also be a pest in this plant because they produce scratches on the leaves of *D. mollis* seedlings and therefore cause chlorosis and premature fall of leaves. The preferred treatment for the production of *D. mollis* seedlings is the control condition (without fertilization) because it showed higher levels of flavonoids than did other treatments and because it did not result in higher insect numbers.

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