

## **DO SALICYLIC ACID, NITRIC OXIDE AND FEEDING BY *MAHANARVA SPECTABILIS* NYMPHS INDUCE A RESISTANCE RESPONSE IN ELEPHANT GRASS?**

By M. V. LEITE†, A. M. AUAD†‡, T. T. RESENDE†, M. P. FRIAS†,  
M. G. FONSECA† and R. J. C. CASTRO§

†*Entomology Laboratory, Embrapa Dairy Cattle Research Station, Juiz de Fora, Brazil*  
and §*Federal University of Juiz de Fora, São Pedro, MG, Brazil*

(Accepted 5 November 2013; First published online 16 December 2013)

### SUMMARY

The objective of this study was to determine whether salicylic acid, nitric oxide (NO) and/or feeding by nymphs of the spittlebug *Mahanarva spectabilis* affect the concentration of total phenolic compounds and the dry matter content of different genotypes of elephant grass (*Pennisetum purpureum*). Four genotypes of elephant grass with varying levels of resistance to insect attack were treated with 4 mL of salicylic acid (1%) or 2 mL of NO per plant in the presence and the absence of nymphs. We determined the concentration of total phenolic compounds and the percentage dry matter of shoots and roots in the plants. We found that salicylic acid, NO and attack by *M. spectabilis* did not change significantly the total phenolic compounds concentration in any of the genotypes of elephant grass tested. However, we observed variations in the concentration of phenolic compounds produced between the resistant and susceptible genotypes, both in the shoot, when subjected to salicylic acid or nymphs, and in the roots in the presence of nymphs. Furthermore, the inducers resulted in variation in the dry matter content of the shoots and roots for most of the genotypes tested. Therefore, we conclude that chemical inducers and feeding by *M. spectabilis* nymphs cannot be used to elicit a resistance response in elephant grass by stimulating the production of phenolic compounds.

### INTRODUCTION

Elephant grass, *Pennisetum purpureum*, is considered as one of the most important forage species in Brazil due to its high potential for dry matter production, nutritional quality, palatability, vigour and persistence (Pereira *et al.*, 2001). However, spittlebug attacks on this specie cause severe damage and reduce its availability to cattle, resulting in significant economic loss. Since the chemical control of spittlebugs is not economically feasible, the induction of resistance in elephant grass needs to be considered as an alternative control measure.

Resistance is induced by activating multiple plant defence pathways, which can lead to changes in the quality and quantity of secondary metabolic compounds and defence proteins (Vendramim and França, 2006). As part of this process, there is an increased activity of peroxidase and the phenylalanine ammonia-lyase, as well as an increased concentration of phenolic compounds, which are effective against a variety

‡Corresponding author. Email: amauad@cnpgl.embrapa.br

of herbivorous insects on a given plant species (Thaler *et al.*, 2001). Plants have the ability to produce toxic or repellent secondary metabolites as direct defences, and volatile molecules, which play an important role in indirect defence (Kessler and Baldwin, 2002). The production of these compounds by plants carries a metabolic cost; however, the balance between induced and constitutive defences can be altered by manipulating plant genotypes and the environment (Gatehouse, 2002).

One of the main demands of milk producers of Brazil refers to the obtaining of elephant grass cultivars resistant to *M. spectabilis*. To date, the elephant grass genotypes selected as resistant to this insect pest allows nymphal survival above 50% (Auaud *et al.*, 2007), thereby justifying the use of chemical inducers for increased resistance in these genotypes. The salicylic acid and nitric oxide (NO) are viable options, since these substances can play an important role in the activation of defence responses of plants to biotic stresses (Klessig *et al.*, 2000, Simaei *et al.*, 2011). In addition, higher concentrations of some phenolic compounds can be induced in plants by insect feeding (Karban and Baldwin, 1997).

Thus, the present study aimed to investigate whether salicylic acid, NO and/or feeding by nymphs of the spittlebug *Mahanarva spectabilis* alter the concentration of phenolic compounds and the dry matter content of different genotypes of elephant grass.

#### MATERIAL AND METHODS

We evaluated four genotypes of elephant grass, which differed in their resistance to insect attack as follows: Cameroon de Piracicaba (resistant), Roxo de Botucatu (susceptible) and Sem Pêlo and Cameroon (intermediate resistance), following the classification by Auaud *et al.* (2007). The plants were obtained from the forage breeding programme of Embrapa Dairy Cattle, planted in 500-mL pots, and maintained in a greenhouse for 35 days. The inducers applied to plants of each genotype were salicylic acid, NO and *M. spectabilis* nymphs. The chemical inducers were used to promote a defence response in plants under biotic stress (Simaei *et al.*, 2011). In addition, NO promotes normal growth and development of plants at lower concentrations (Beligni and LaMattina, 2001), and salicylic acid is analogous to the commercially used resistance inducer acibenzolar-S-methyl (ASM-Bion<sup>®</sup>).

For the salicylic acid treatment, 4 mL of 1% salicylic acid solution was applied to the soil in each 500-mL pot two days after the start of the experiment; control plants received 4 mL of distilled water. For the NO treatment, the shoots of each plant were sprayed with 2 mL of 0.15-g/L Sodium Nitroprussiate solution two days after the start of the experiment, and this treatment was repeated every three days; control plants were sprayed with distilled water following the same procedure.

The *M. spectabilis* nymphs, which were used to verify the induction of resistance, were collected from the experimental fields of Embrapa Dairy Cattle and maintained on elephant grass plants in a greenhouse. For the feeding experiment, four nymphs of the 4th or 5th instars from the rearing stock were placed in 500-mL pots, which was covered by a lid containing a central hole to allow the passage of shoots; a layer of gauze was placed around the hole to prevent the escape of spittlebugs. The nymphs

remained on the plants for 10 days, and any nymphs that died or emerged as adults were replaced daily to maintain the density of insects.

Ten days after the application of each treatment, the shoots from each plant ( $n = 120$  plants) were cut and weighed to determine fresh weight. The roots were washed with water jets, placed on paper for 24 hours to remove excess water and weighed to determine the fresh weight. The shoots and roots were then put in an oven heated to 55 °C until their weight stabilized to determine dry weight. The dry matter was grounded and each sample was individualized and identified for biochemical analysis. Phenolic compounds were extracted from the samples using acetone (70%). These were put in the Folin-Denis reagent, and concentrations of phenols present in plants subjected to the different treatments were determined using a spectrophotometer at 760 nm.

The assays were conducted in a randomized block design in a factorial arrangement ( $4 \times 6$ ). We used four elephant grass genotypes (Cameroon de Piracicaba, Sem Pêlo, Cameroon and Roxo de Botucatu) with six inducers (spittlebug nymphs, application of salicylic acid or NO with and without nymphs and a control) with five replications of each treatment.

We investigated the effect of each chemical inducer in the presence and absence of *M. spectabilis* nymphs, and the effect of feeding by the nymphs alone on the concentration of total phenolics and the dry matter content of shoots and roots produced by different genotypes of elephant grass. The data were analysed using analysis of variance, and mean values were compared using the Scott–Knott test at a significance level of 5%.

## RESULTS AND DISCUSSION

In the absence of inducers (control), we observed no significant variation in the concentration of phenolic compounds in the shoot between the four genotypes of elephant grass tested (Table 1), indicating that there is no relationship between the degree of resistance and the concentration of phenolic compounds for these genotypes. In contrast, Silva *et al.* (2005) observed differences in the concentration of phenolic compounds in the leaves of different genotypes of sugarcane in the absence of inducers, with the highest concentration being found in the resistant cultivar; the authors also observed that this cultivar is not often infested under field conditions, which they attributed to the high concentration of phenolic compounds. Similarly, Guimarães *et al.* (2008) found a higher concentration of phenolic compounds in the resistant cultivar of sugarcane, indicating that resistance arises from antibiosis. According to Auad *et al.* (2007), the resistant cultivar used in our study, Cameroon de Piracicaba, also has resistance by antibiosis; however, it is possible that this resistance is related to a characteristic other than the concentration of phenolic compounds, since, in the present study, the concentration of phenolic compounds was the same in resistant and susceptible plants.

In the presence of *M. spectabilis* nymphs or salicylic acid, the concentration of phenolic compounds was higher in genotype Roxo de Botucatu (susceptible) than other genotypes (Table 1). For the other inducers, the concentration of phenolic compounds

Table 1. Total phenolic compounds concentration (ppm) and dry matter (%) of shoot of elephant grass genotypes submitted to different inducers of resistance.

Treatment	Elephant grass genotypes				F	<i>p</i>
	Cameroon de Piracicaba	Sem Pêlo	Cameroon	Roxo de Botucatu		
	Phenol concentration (ppm)					
Control	5.87 ± 1.18 Aa	5.12 ± 1.14 Aa	6.49 ± 1.48 Aa	7.06 ± 0.90 Aa	1.31	0.2785
Nymph	3.70 ± 0.65 Ab	4.69 ± 0.73 Ab	5.02 ± 0.69 Ab	8.08 ± 0.35 Aa	5.47	0.0020
AS	5.54 ± 1.70 Ab	6.15 ± 1.40 Ab	6.20 ± 1.11 Ab	8.15 ± 1.30 Aa	2.43	0.0423
AS + nymph	5.15 ± 0.74 Aa	7.07 ± 1.29 Aa	7.01 ± 1.18 Aa	7.40 ± 1.54 Aa	1.97	0.1261
NO	4.57 ± 1.00 Aa	6.47 ± 1.26 Aa	5.61 ± 0.91 Aa	7.41 ± 1.12 Aa	2.77	0.0578
NO + nymph	5.00 ± 1.60 Aa	4.77 ± 0.59 Aa	4.39 ± 0.65 Aa	5.87 ± 1.12 Aa	0.74	0.5296
F	1.11	1.74	1.79	1.25		
P	0.3635	0.1381	0.1268	0.2971		
	Dry matter (%)					
Control	23.18 ± 0.25 Aa	21.89 ± 0.20 Aa	19.60 ± 0.33 Bb	19.02 ± 0.74 Ab	8.36	0.0001
Nymph	20.28 ± 0.91 Bb	22.78 ± 0.29 Aa	21.94 ± 1.08 Ba	19.44 ± 1.33 Ab	4.09	0.0099
AS	25.07 ± 0.98 Aa	22.39 ± 0.27 Ab	20.94 ± 1.15 Bc	18.84 ± 0.23 Ad	15.05	0.0001
AS + nymph	24.71 ± 0.74 Aa	23.41 ± 0.63 Aa	21.55 ± 0.95 Bb	19.33 ± 0.17 Ac	12.07	0.0001
NO	23.95 ± 0.59 Aa	23.47 ± 0.70 Aa	22.47 ± 0.21 Aa	19.34 ± 0.39 Ab	9.48	0.0001
NO + nymph	23.30 ± 0.80 Aa	23.95 ± 0.55 Aa	24.14 ± 0.57 Aa	19.07 ± 0.34 Ab	12.59	0.0001
F	6.43	1.29	5.10	0.11		
P	0.0001	0.2774	0.0005	0.99		

AS = Salicylic acid; NO = Nitric oxide.

Mean values followed by the same small letter in the row and the same capital letter in the column are not significant (ANOVA followed by a Scott–Knott test,  $p \leq 0.05$ ).

did not differ between genotypes. Silva *et al.* (2005) have previously noted that the concentration of phenols was higher in leaves of sugarcane infested with *M. fimbriolata*, but this difference was only significant at higher insect densities. Likewise, in the present study, a density of four nymphs per plant was enough to trigger the defence mechanisms of susceptible plants.

Within each elephant grass genotype, none of the treatments tested had a significant effect on the concentration of phenolic compounds in shoots (Table 1). Thus, it can be concluded that although salicylic acid, NO (Klessig *et al.*, 2000) and insect feeding (Karban and Baldwin, 1997) are important in the activation of plant defence mechanisms, they did not act systemically in elephant grass.

Although none of the inducers promoted a change in the concentration of phenolic compounds in the shoots within the genotypes, it was found that there was a significant increase in the dry matter content of the genotype Cameroon de Piracicaba (resistant) in the presence of salicylic acid and NO with or without the nymph, and the genotype Cameroon (intermediate) in the presence of NO with or without the nymph (Table 1). According to Simaei *et al.* (2011), salicylic acid and NO act as regulators of plant growth, and they found that the combination of these inducers resulted in greater leaf area, and shoot fresh and dry weights than either of them alone. Thus, it can be

Table 2. Total phenolic compounds concentration (ppm) and dry matter (%) of root of elephant grass genotypes submitted to different inducers of resistance.

Treatment	Elephant grass genotypes				F	<i>p</i>
	Cameroon de Piracicaba	Sem Pêlo	Cameroon	Roxo de Botucatu		
	Phenol concentration (ppm)					
Control	1.76 ± 0.42 Ab	1.91 ± 0.11 Ab	2.76 ± 0.25 Aa	3.16 ± 0.22 Aa	4.95	0.0031
Nymph	1.30 ± 0.35 Ab	1.89 ± 0.36 Ab	2.64 ± 0.24 Aa	3.03 ± 0.33 Aa	5.76	0.0012
AS	1.39 ± 0.47 Aa	1.58 ± 0.48 Aa	2.06 ± 0.60 Aa	2.30 ± 0.52 Aa	1.92	0.1315
AS + nymph	1.77 ± 0.39 Aa	1.77 ± 0.56 Aa	2.30 ± 0.68 Aa	2.54 ± 0.58 Aa	1.55	0.2063
NO	2.04 ± 0.23 Aa	1.99 ± 0.17 Aa	2.16 ± 0.24 Aa	2.25 ± 0.53 Aa	0.15	0.9303
NO + nymph	2.32 ± 0.22 Aa	1.52 ± 0.45 Aa	2.43 ± 0.10 Aa	2.38 ± 0.64 Aa	1.72	0.1672
F	1.52	0.36	0.81	1.67		
P	0.1901	0.8775	0.5498	0.1502		
	Dry matter (%)					
Control	67.66 ± 4.38 Aa	50.15 ± 1.97 Ba	30.31 ± 0.76 Bb	35.79 ± 4.76 Bb	7.33	0.0002
Nymph	58.89 ± 4.89 Aa	43.12 ± 8.90 Bb	33.42 ± 2.29 Bb	30.89 ± 1.49 Bb	3.61	0.0151
AS	61.66 ± 10.78 Aa	68.86 ± 11.51 Aa	61.06 ± 9.58 Aa	33.03 ± 3.30 Bb	6.58	0.0005
AS + nymph	75.24 ± 6.31 Aa	50.97 ± 10.33 Bb	52.82 ± 8.79 Ab	27.60 ± 5.44 Bc	9.96	0.0001
NO	57.85 ± 8.87 Aa	56.60 ± 6.07 Aa	49.99 ± 5.24 Aa	55.09 ± 7.70 Aa	0.31	0.8157
NO + nymph	44.64 ± 2.00 Aa	33.93 ± 6.40 Ba	35.91 ± 2.57 Ba	37.35 ± 2.68 Ba	0.54	0.6529
F	2.78	3.32	4.00	2.40		
P	0.222	0.0084	0.0024	0.0383		

AS = Salicylic acid; NO = Nitric oxide.

Mean values followed by the same small letter in the row and the same capital letter in the column are not significant (ANOVA followed by a Scott–Knott test,  $p \leq 0.05$ ).

inferred that the simultaneous application of inducers would have promoted a greater increase in dry mass than that obtained in the present study.

Among the genotypes, it was found that dry matter content was lower in the susceptible cultivar than in the resistant cultivar, both in the control treatment and in those in which were applied chemical inducers alone or together with nymphs of *M. spectabilis* (Table 1). It is noteworthy that the application of NO in both presence and absence of nymphs increased the dry matter content of plants with an intermediate level of resistance to a level equaling that observed in the resistant cultivar, suggesting that the use of NO may be promising for cultivars, Cameroon and Sem Pêlo.

Within each genotype none of the treatments had a significant effect on the concentration of phenolic compounds in the roots, which are the feeding sites of *M. spectabilis* nymphs (Table 2). These results are contrary to those of Silva *et al.* (2005), who found that infestation by the spittlebug *M. fimbriolata* led to an increase in phenolic compounds in sugarcane. It is likely that this difference is due to the number of nymphs used in the present study being insufficient to promote a response in the host plant. Also, the quantification of total phenolic compounds, as opposed to individual compounds, may have prevented us from detecting variation in the concentration of specific phenols in the roots in response to the inducers. According to Nutt *et al.* (2004), the types and quantities of phenolic compounds vary in the roots of sugarcane

when attacked by Scarabaeidae; these authors claimed that some of the phenolic compounds that decreased in concentration were probably precursors of some that increased. This highlights the importance of qualifying and quantifying phenols to accurately measure the performance of inducers.

There was also no significant difference in the concentration of total phenolics in the roots between the four genotypes when subjected to the chemical inducers, salicylic acid and NO, in the presence and absence of *M. spectabilis* nymphs. However, in the absence of inducers (control) and where only spittlebug nymphs were present, the concentration of total phenolics was lower in the resistant genotype Cameroon de Piracicaba than in the susceptible genotype Roxo de Botucatu (Table 2). This result contrasts with that of Guimarães *et al.* (2008), who found the highest amount of phenols in the roots of the resistant cultivar for sugarcane. Similarly, Ghumare and Mukherjee (2003) and Beninger *et al.* (2004) found that plants that accumulate high levels of phenols tend to inhibit the development of herbivores. However, the same was not observed in this study, since the genotype Roxo de Botucatu, despite being susceptible to insect attack (Aquad *et al.*, 2007), showed a higher concentration of phenols.

It is also worth mentioning that for the control treatment and in the presence of nymphs, the concentration of total phenolics and dry matter content for the cultivars Sem Pêlo and Cameroon were equal to levels found in the resistant and susceptible genotypes, respectively.

Within genotype Cameroon de Piracicaba, the dry matter content of the roots was not affected by the presence of inducers (Table 2). However, for genotype Roxo de Botucatu, the application of NO alone resulted in an increased dry matter content of the roots. Nitric oxide and salicylic acid alone promoted the same effect in the Sem Pêlo cultivar. For genotype Cameroon, both NO alone and salicylic acid in the presence or absence of nymphs significantly increased the dry matter content of the roots. When comparing between genotypes, the dry matter content was always higher in the resistant cultivar in plants infested with *M. spectabilis* nymphs and/or to which salicylic acid was applied. In contrast, there was no significant difference between genotypes in the dry matter content of plants to which NO was applied in the presence or absence of nymphs.

#### CONCLUSIONS

The salicylic acid, NO and attack by *M. spectabilis* cannot be used to elicit a resistance response in elephant grass by stimulating the production of phenolic compounds.

Variations were observed in the concentration of phenolic compounds produced between the resistant and susceptible genotypes, both in the shoots, when subjected to salicylic acid or nymphs, and in the roots in the presence of nymphs.

The inducers resulted in variation in the dry matter content of the shoots and roots for most of the genotypes tested, and so may be of use in this plant species for this purpose.

*Acknowledgements.* To FAPEMIG, the UNIPASTO and CNPq for the financial support of this research.

## REFERENCES

- Auad, A. M., Simões, A. D., Pereira, A. V., Braga, A. L. F., Sobrinho, F. S., Léo, F. J. S., Paula-Moraes, S. V., Oliveira, S. A. and Ferreira, R. B. (2007). Seleção de genótipos de capim-elefante quanto à resistência à cigarrinha-das-pastagens. *Pesquisa Agropecuária Brasileira* 42:1077–1081.
- Beligni, M. V. and Lamattina, L. (2001). Nitric oxide in plants: the history is just beginning. *Plant Cell Environmental* 24:267–278.
- Beninger, C. W., Abou-Zaid, M. M., Kistner, A. L., Hallett, R. H., Iqbal, M. J., Grodzinski, B. and Hall, J. C. (2004). A flavanone and two phenolic acids from *Chrysanthemum mor. folium* with phytotoxic and insect growth regulating activity. *Journal of Chemical Ecology* 30:589–606.
- Gatehouse, J. A. (2002). Plant resistance toward insect herbivores: a dynamic interaction. *New Phytologist* 156:145–169.
- Ghumare, S. S. and Mukherjee, S. N. (2003). Performance of *Spodoptera liturata* Fabricius on different host plants: influence of nitrogen and total phenolics of plants and mid-gut esterase activity of the insect. *Indian Journal of Experimental Biology* 41:895–899.
- Guimarães, E. D., Mutton, M. A., Ferro, M. I. T., Silva, J. A. and Mutton, M. J. R. (2008). Níveis constitutivos de compostos fenólicos podem estar relacionados à resistência da cana-de-açúcar às cigarrinhas-das-raízes. *Revista de Agronegócios e Meio Ambiente* 1:357–365.
- Karban, R. and Baldwin, I. T. (1997). *Induced Response to Herbivory*. Chicago, IL: University of Chicago Press, 330 pp.
- Kessler, A. and Baldwin, I. T. (2002). Plant responses to insect herbivory: the emerging molecular analysis. *Annual Review Plant Biology* 53:299–328.
- Klessig, D. F., Durner, J., Noad, R., Navarre, D. A., Wendehenne, D., Kumar, D., Zhou, J. M., Shah, J., Zhang, S., Kachroo, P., Trifa, Y., Pontier, D., Lam, E. and Silva, H. (2000). Nitric oxide and salicylic acid signaling in plant defense. *Proceedings of the National Academy of Sciences* 97:8849–8855.
- Nutt, K. A., O'shea, M. G. and Allsopp, P. G. (2004). Feeding by sugarcane whitegrubs induces changes in the types and amounts of phenolics in the roots of sugarcane. *Environmental and Experimental Botany* 51:155–165.
- Pereira, A. V., Do Valle, C. B., Ferreira, R. D. P. and Miles, J. W. (2001). Melhoramento de Forrageiras Tropicais. In *Recursos Genéticos e Melhoramento de Plantas*, 549–602 (Eds L. L. Nass, A. C. C. Valois, I. S. Melo and M. C. Valadares-Ingliš). Rondonópolis, Brazil: Fundação Mato Grosso.
- Silva, R. J. N., Guimarães, E. R., Garcia, J. F., Botelho, P. S. M., Ferro, M. I. T., Mutton, M. A. and Mutton, M. J. R. (2005). Infestation of froghopper nymphs changes the amounts of total phenolics in sugarcane. *ScientiaAgricola* 62:543–546.
- Simaei, M., Khavari-Nejad, R. A., Saadatmand, S., Bernard, F. and Fahimi, H. (2011). Effect of salicylic acid and nitric oxide on antioxidant capacity and proline accumulation in *Glycine max* L. treated with NaCl salinity. *African Journal of Agricultural Research* 6:3775–3782.
- Thaler, J. S., Stout, M. J., Karban, R. and Duffey, S. S. (2001). Jasmonat-mediated induced plant resistance affects a community of herbivores. *Ecological Entomology* 26:312–324.
- Vendramim, J. D. and França, S. C. (2006). Indução de resistência a insetos. In *Indução de resistência em plantas a patógenos e insetos*, 11–28 (Eds L. S. Cavalcanti, R. M. Di Piero, P. Cia, S. F. Pascholati, M. L. V. Resende and R. S. Romeiro). Piracicaba, Brazil: Editora FEALQ.