# CROP BREEDING AND APPLIED BIOTECHNOLOGY

# NOTE

# Lack of antibiosis against *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae) in *Cenchrus purpureus* (Schumach.) Morrone germplasm

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**Abstract:** This study examines Cenchrus purpureus with the aim of identifying genotypes and select plants progenitors resistant to Mahanarva spectabilis (Distant, 1909) for future recurrent selection from the elephant grass germplasm bank. Six M. spectabilis eggs were inserted into each plant of 138 elephant grass genotypes. After 35-45 days the percentage of nymphal survival was assessed. Despite the large variation in insect survival there were no significant differences in the joint analyses of bioassays conducted from 2008 to 2024. None genotype yielded an insect survival rate of less than 30%, and fewer than 10% of the genotypes showed a survival rate of less than 50%. These genotypes should be intercrossed, forming a new population each year, with the aim of increasing the presence of favorable alleles for resistance to this insect pest, with the goal of producing genotypes that achieve future M. spectabilis nymph mortality greater than 70%.

Keywords: Elephant grass, spittlebugs, pastures

# INTRODUCTION

Tropical forage plants are of great importance for Brazilian livestock farming, being cultivated for grazing on approximately 177 million hectares (Bolfe et al. 2024) and capable of increasing the productive potential of pastures and consequently animal production (Martha et al. 2012, Simeão et al. 2016). Among these, elephant grass, *Cenchrus purpureus (Schumach.)* Morrone is an important forage in many tropical and subtropical regions because of its high yield, nutritional value, acceptability to animals, vigor and persistence. This grass is widely used and can be grazed directly in the form of chopped green grass, hay or silage for animal feed. It is also a potential crop for producing bioenergy (Silva et al. 2018, Pereira et al. 2021).

Monocropping this forage can induce an outbreak of the spittlebug *Mahanarva spectabilis* (Distant 1909) (Hemiptera: Cercopidae), causing a decline in the production of foliage biomass (Auad et al. 2007). This insect pest is economically important in the American tropics and subtropics. It attacks a variety of tropical forages, resulting in losses of nearly US\$ 2.1 billion a year (Thompson 2004).

Selecting forage plants that are resistant to spittlebug nymphs has proven to be an efficient and economically viable control strategy (Cardona et al. 1999).

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<sup>1</sup> Embrapa Gado de Leite, Avenida Eugênio do Nascimento, 610, Aeroporto, 36038-330, Juiz de Fora, MG, Brazil The biological performance of insects can be compromised by the resistance mechanisms present in plants. Know and improve action of this mechanisms can promote reduction in pest populations (Valério 2009).

Considering the importance of developing techniques for selecting forage cultivars resistant to the nymphs of *M*. *spectabilis*, the aims of this study were to identify genotypes and select plants progenitors resistant to *M*. *spectabilis* for future selection in *C*. *purpereus* germplasm bank.

### MATERIAL AND METHODS

A total of 138 elephant grass accessions (genotypes) from the Embrapa Germplasm Bank (GB) were evaluated. Mature tillers were obtained from each and cut into pieces, containing two buds. These cuttings were planted in 0.5 L pots containing a 1:1 mixture of soil and manure. The pots were kept in a greenhouse, with an average temperature of 27.5 °C and 75% humidity. Automatic irrigation was used three times a day for 15 minutes. After the rooting and sprouting of the cuttings, fertilization was carried out every two weeks with 30 mL of a mixture containing 30 g of urea, 12 g of potassium chloride and 12 g of simple superphosphate per liter of water. After reaching a height of 30 cm, the plants were prepared for the insertion of *M. spectabilis* eggs.

Adults of *M. spectabilis* were collected from Coronel Pacheco, Minas Gerais State and Valença, Rio de Janeiro State, taken to the Entomology Laboratory of Embrapa Dairy Cattle, and placed in acrylic cages. Inside each cage an elephant grass plant (after reaching a height 60 cm) was added as a feeding substrate and maintained for 5 to 7 days. The bottoms of the cages were covered with hydrophilic gauze that served as a substrate for oviposition. The eggs deposited by the adults on the gauze were washed in running water over sieves, and those retained on the 400 mesh were placed in 9 cm diameter Petri dishes lined with filter paper, where a 1% copper sulfate solution was added to avoid fungal contamination. The plates containing the eggs were then transferred to a conditioned chamber at  $28 \pm 2$  °C, 14 hours of photophase and a relative humidity of 70 ± 10% until they reached the embryonic stage close to hatching (S4).

Six eggs of *M. spectabilis* at the S4 stage were inserted into each plant (0.5 L pot) of the 138 elephant grass genotypes. The pots were then closed with a plastic lid with a circular opening around the stem of the plant. The opening was closed with hydrophilic gauze approximately 40 cm long  $\times$  10 cm wide to ensure that the nymphs remained in the pots.

After 35-45 days that the eggs were inserted in the plant the nymphs were classified as small (first and second instars), medium (third instars) or large (fourth and fifth instars) and counted, and for those that were medium and large the percentage of nymphal survival was assessed.

The experiments were conducted in a completely randomized block design with fifteen replications. The percentage of nymphal survival was evaluated each year (2008, 2011, 2012, 2019 and 2024) with different number of elephant grass genotypes in order to test a total of 138 elephant grass genotypes from the Embrapa GB. A joint analysis was carried out using the averages of each genotype in each year, adopting Federer's augmented block design and considering the Roxo de Botucatu and Pioneiro cultivars as the controls' treatments for all years/trial. The Statistical analyses and estimates genetic and phenotypic parameters were carried out with Genes software (Cruz 2008).

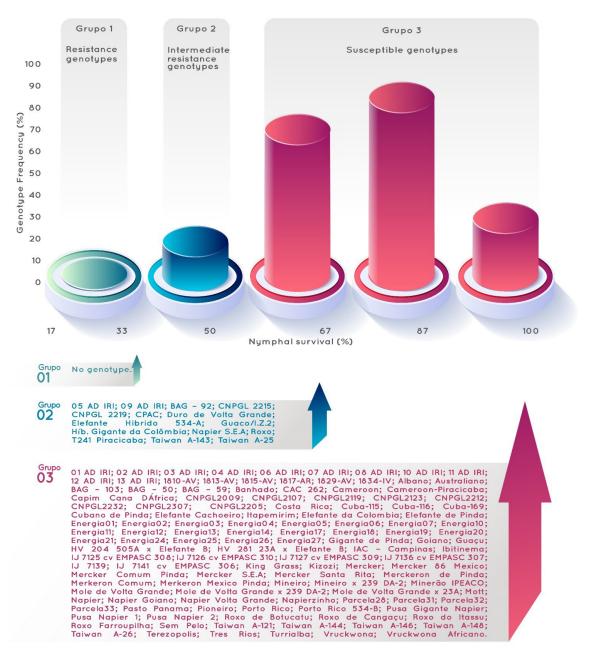
A graphical analysis of the percentage of genotypes associated with nymphal survival in the range of 1 (17%), 2 (33%), 3 (50%), 4 (67%), 5 (83%), and 6 (100%) was carried out for the experiments between 2008 and 2024. The frequency of genotypes was divided into three groups of plants: one of plants whose nymphal survival was equal to or less than 33%, a second with nymphal survival between 34% and 50%, and the last with nymphal survival greater than 50%, following the classification adapted by (Cardona et al. 1999) for resistant, intermediate resistant and susceptible genotypes.

#### **RESULTS AND DISCUSSION**

The average nymphal survival of *M. spectabilis* ranged from 36 to 98% among the 138 genotypes evaluated between 2008 and 2024. Despite the large variation in insect survival there were no significant differences (P=1.88 and F = 21.45) in the joint analyses. None genotype had an insect survival rate of less than 30%, and fewer than 10% of them had a survival rate of less than 50%. These results show that the vast majority of elephant grass genotypes bank are indeed susceptible to *M. spectabilis* (Figure 1).

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On the other hand, the estimated heritability for the survival of insect nymphs of *M. spectabilis* in elephant grass genotypes was 60.1%, considered moderate to high, indicating that this trait can be successfully passed on to offspring plants. Thus, selecting genotypes that are less susceptible to insects, i.e., those with lower survival rates for *M. spectabilis* nymphs, could be an interesting strategy for obtaining resistant elephant grass genotypes in breeding programs. The estimate of the ratio between the genetic and environmental coefficients of variation (CVg/CVe), which is greater than unity (CVg/CVe = 1.23), reinforces this claim.



*Figure 1.* Average nymphal survival (%) of *Mahanarva spectabilis* in 138 *Cenchrus purpereus* genotypes and the frequency distribution of the tested genotypes (%) with nymphal survival ranging from 0 to 33% (resistant), 34 to 50% (intermediate resistance) and greater than 50% (susceptible) between trials carried out in 2008, 2011, 2012, 2019 and 2024.

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In studies on the resistance of forage to spittlebugs, a plant is considered resistant when the survival rate of the insect pest is less than 30% (Cardona et al. 1999). In this study, conducted between 2008 and 2024, 100% of the evaluated genotypes showed nymphal survival rates greater than 30%, indicating that the genotypes of this forage do not have deleterious effects on the immature stage. These are characterized as susceptible to *M. spectabilis* and corroborate those results of Auad et al. (2007), Alvarenga et al. (2017), Alvarenga et al. (2019), Perez et al. (2019).

Owing to the genetic limitations associated with resistance to *M. spectabilis* within elephant grass genotypes bank, the introduction of new accessions is an alternative for identifying sources of resistance. Although this strategy is the most recommended, it has long-term repercussions because of the importation of genetic material, initial cultivation and multiplication within the germplasm and genetic characterization for later use in breeding. In the medium term, strategies involving the use of the genetic resources available in the genotypes bank are the most viable. The use of phenotypic recurrent selection, for example, is a strategy that could be used in parallel with breeding for other traits of forage interest or for energy, with the aim of increasing the number of favorable alleles for resistance to spittlebugs. Thus, through consecutive selection cycles, identifying plants with lower survival rates of the pest insect nymphs and intercrossing these plants can hopefully lead to an improved population in each cycle. In these populations, as a result of the gradual increase in the frequency of the alleles responsible for characteristics that are unfavorable to the development of insects, it will be easier to identify plants with lower pest insect survival rates.

The estimated heritability for resistance to spittlebugs was moderate to high and the ratio between the genetic and environmental coefficients of variation was greater than unity, favoring the process of selecting superior individuals to successfully form improved populations. This suggest that recurrent selection for resistance to spittlebugs be adopted within the elephant grass breeding program. So, initially, it recommends to use the genotypes of this forage plant with average nymphal survival rates less than 50% (Figure 1) (Guaco/I.Z.2, Roxo, Híbrido Gigante da Colômbia, Elefante Híbrido 534-A, T247 Piracicaba, Roxo de Cangaçu, BAG-92, CPAC, Duro de Volta Grande, 05 AD IRI, Taiwan A-25, C2215, 09 AD IRI, Napier S.E.A.). These materials will have to be crossed with each other, or even self-fertilized, to obtain a population with great variability in which individuals will be selected that, at each selection cycle, will decrease the survival of *M. spectabilis*. These genotypes will be intercrossed, forming a new population each year with the aim of increasing the presence of favorable alleles for resistance to the insect pest and thus to obtain genotypes that increase future mortality in *M. spectabilis* nymphs to levels greater than 70%.

As recurrent selection is a highly dynamic strategy, the introduction of new genetic materials from possible accessions from the genotypes bank, for example, is feasible at any time without harming the program. Similarly, the plants selected for tolerance or resistance to spittlebugs in each cycle can be used as parents in crosses aimed at obtaining cultivars that combine forage or energy characteristics with resistance to the pest. Examples of the successful use of recurrent selection in different variations are frequent in the literature (Miles et al. 2006, Souza Sobrinho et al. 2010, Mateus et al. 2015). Resende et al. (2024), evaluating the success of the *Urochloa ruziziensis* genetic improvement program for resistance to spittlebugs, detected considerable gains with phenotypic recurrent selection. Starting from a susceptible population with an average nymphal survival of 75% over 16 years, it was possible to obtain an improved population in which 60% of the plants showed nymphal survival of less than 33% and were therefore considered resistant to the insect pest. The overall average survival rate of this improved population was 32% for the nymphs of *M. spectabilis* and *D. schach.* 

According to Pereira et al. (2008), the analysis of the genetic distance between the access groups of elephant grass Porto Rico, Santa Rita, IJ-7136 cv. Empasc 307, Mineiro and Mineiro Ipeaco revealed a zero genetic distance, suggesting that these materials are identical although they have different names. In addition, certain pairs of accessions, such as Napier and Mineiro or Mineiro Ipeaco; P 241 Piracicaba and Guaçu/IZ2; Capim Cana D'África and Vrukwona; Merker Comum and Merker Comum de Pinda, presented minimal genetic distance. This genetic proximity suggests a close relationship among the above genotypes. The aforementioned genotypes are among those used in our antibiosis experiments against *M. spectabilis*. Thus, the genetic proximity and even the presence of duplicates within of elephant grass genotypes bank, as evidenced by Pereira et al. (2008), may explain the similar behavior of the elephant grass genotypes in terms of resistance to *M. spectabilis*. Notably, for most of the important forage characteristics, there is a great deal of genetic variability in the accessions in Embrapa Gado de Leite's elephant grass genotypes bank. This variability has been exploited by genetic improvement and has culminated in the launch of several cultivars that have contributed greatly to national livestock farming such as Kurumi and Capiacu cultivars (Pereira et al. 2017). Lack of antibiosis against Mahanarva spectabilis (Distant) (Hemiptera: Cercopidae) in Cenchrus purpureus (Schumach.)...

Importantly, genetic diversity or variability is the basis for identifying and selecting more resistant plants; i.e., without genetic diversity or variability, there is no genetic improvement. Taking advantage of this variability makes it possible to identify genotypes that can respond to abiotic and biotic stresses in different environments (Ivoglo et al. 2008). Therefore, maintaining a diversified genotypes bank is fundamental to defining an effective strategy for selecting plants progenitors for improvement programs. Notably, the genotypes of the elephant grass germplasm showed little genetic divergence in terms of resistance to *M. spectabilis*, thus limiting strategies for obtaining cultivars resistant to spittlebugs. Amabile et al. (2018) stated that difficulties in plant breeding can be caused by a lack of genetic resources, with a limited number of available sources of resistance against pests and diseases. This narrow genetic base for breeding studies is a constant concern among breeders and has already been reported for different crops, such as soybeans (Yamanaka et al. 2007, Priolli et al. 2010, Wysmierski and Vello 2013).

# CONCLUSION

It was found lack of antibiosis against Mahanarva spectabilis in Cenchrus purpureus germplasm.

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# DATA AVAILABILITY

The datasets generated and/or analyzed during this research are available from the corresponding author upon request.

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