Acta Scientiarum

http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v44i1.56622

PASTURE AND FORAGE UTILIZATION

Components of seed production and ergot resistance used as criteria for selection of Brachiaria hybrids

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ABSTRACT. Ergot is a fungal disease and causes reductions in seed productivity and quality. We aimed to identify promising genotypes of brachiaria evaluating: (1) seed production potential (number of tillers, racemes per inflorescence, spikelets per raceme, raceme length, weight and number of seeds, and germination) and (2) resistance of seeds to Ergot (infected tillers, infected seeds). Five genotypes of Brachiaria grasses, four non-natural hybrids named BH1619, BH1810, BH1516 and Mavuno Palisadegrass (*Urochloa brizantha* x *Urochloa ruziziensis*), and Marandu Palisadegrass (*U. brizantha* Hoechst cv. Marandu) were evaluated. The BH1619 hybrid, despite the high weight of seeds, produced less viable, pure seeds. The hybrid BH1516 had a lower percentage of flowered tillers (42%) and seeds infected with ergot (8%). Marandu Palisadegrass had a higher percentage of infected tillers (95%) and infected seeds (38%). The hybrid BH1516 was the most resistant to ergot, followed by Mavuno Palisadegras sand BH1619. Among all materials, Mavuno Palisadegrass and BH1516 have a higher potential for seed production due to the higher percentage of flowered tillers and production of pure seeds with high germination capacity. The BH1516 hybrid was resistant to ergot, making it a better choice for use in infected areas used for seed or forage production.

Keywords: *Claviceps* spp.; flowering; germination; grass seed production; tiller.

Received on November 11, 2020.
Accepted on September 9, 2021.

Introduction

The tight genetic base of cultivated Brachiaria grasses, with apomictic reproduction, results in extensive monoculture of pastures. Such characteristic facilitates the emergence and propagation of pests and diseases, causing considerable losses to seed production. For this reason, in Brazil the extensive cultivated areas with Brachiaria grasses present high risk of genetic vulnerability. The use of new hybrids of Brachiaria grasses results in lower risk of pest and disease spread, better use of different types of soils and rational utilization by several animal categories (Jank, Valle, & Resende, 2011). Higher availability of seeds in number and quality is a determining factor for the choice of forage grasses to be commercially exploited.

Among the most important diseases of Brachiaria grasses, ergot of seeds attacks the inflorescence of grasses (Miedaner & Geiger, 2015) and is widely disseminated in Brazil (Marchi, Fernandes, Anache, & Fabris, 2008). The ergot-causing fungus of Brachiaria seeds was initially described as *Claviceps sulcata* Langdon, a perfect or teleomorphic *Sphacelia* sp. form, of African origin, which affects the ovaries of flowers and causes pollen infertility (Rosatti, Silva, Silva, & Kruppa, 2006; Marchi et al., 2008). rDNA sequence analysis of Brazilian isolates of the pathogenic fungus in *B. brizantha* demonstrated high similarity with *C. maximensis* (Pazoutová, 2002; Marchi et al., 2008). Molecular studies confirmed the identification of *C. maximensis* and Brachiaria grasses in Brazil (Chaves, Gomes, Marriel, & Pfenning, 2004), and recent studies show that 50% commercial seed lots of *B. brizantha* cv. Piatã collected in 10 seed production fields (two seed production fields in each state of Brazil: SP, GO, BA, MG and MS) were infected with more than one species of *Claviceps* (Silva, Martins, Nascimento, Barreto, & Farias, 2019). The association of pathogens to seeds can affect plant establishment, development, seed production, forage persistence in the field and animal production. The presence of these microorganisms also affects the export of forage seeds (Fernandes, Marchi, Jerba, & Borges, 2005), since many countries impose phytosanitary restrictions. The use of fungicides in the soil may reduce the presence of the pathogen (Fernandes, Verzignassi, Mallmann, & Queiroz, 2017; Dung et al., 2018), but the selection of resistant forage grasses would be the most effective management (Miedaner & Geiger, 2015). Considering that Brazil is the leading producer and exporter of tropical seeds of forage grasses in the world (Silva et al., 2019), the commercialization of contaminated seed lots can affect mainly the foreign market, due to restrictions on the presence of *Claviceps* spp. in many countries.

By means of morphological evaluations, we can determine the potential of grass seed production. Since ergot has been one of the most important diseases affecting seed production of Brachiaria grasses in Brazil, the objectives of this work were to identify promising Brachiaria grass hybrids with high seed production potential and ergot resistance, under controlled conditions.

Material and methods

The soil of the experiment (red-yellow Podzolic) was collected in a pasture area (*Instituto de Zootecnia*, Nova Odessa, São Paulo, Brazil) at the 0-20 cm depth, with the following chemical properties: OM: 19 g dm^{-3}, pH $CaCl_2$: 4.8, P (ion-exchange resin extraction method): 4 mg dm^{-3}, K: 0.8 mmol$_c$ dm^{-3},Ca: 16 mmol$_c$ dm^{-3}, Mg: 50 mmol$_c$ dm^{-3}, H + Al: 25 mmol$_c$ dm^{-3}, sum of bases: 21.8 mmol$_c$ dm^{-3},cation exchange capacity: 46.8 mmol$_c$ dm^{-3}, base saturation: 47. The treatments corresponded to four simple and multiple Brachiaria hybrids (BH), intra- and inter-specific apomictic *Brachiaria* spp. named BH1619, BH1810, BH1516 and Mavuno Palisadegrass (*U. brizantha* x *U. ruziziensis*), and one Brachiaria cultivar (*U. brizantha* Hoechst cv. Marandu Syn. *B. brizantha* Hoechst cv. Marandu (Marandu Palisade grass), in a complete randomized block design with five replications. Brachiaria grasses seeds were placed to germinate in Gearbox boxes. After 15 days, ten seedlings of each genotype were transplanted into pots with 5 kg soil. Pots were kept in a greenhouse (Tmax 34°C, Tmin 27°C, relative air humidity 50%), irrigated daily (20 KP field capacity), and thinned to five plants per pot. Nutrient replenishment was applied monthly, 10 mL per pot, diluted in 9 L water: 40 g KH_2PO_4 and 32 g $(NH_4)_2SO_4$. Two experiments were carried out: evaluation of seed production potential, and evaluation of the potential of resistance to ergot. Results were tested by analysis of variance according to the GLM procedure of the statistical package Statistical Analysis System [SAS] (2009). The averages were obtained using the 'LSMEANS' statement and compared by Tukey's Test at the 5% probability level.

Experiment 1: Seed production potential

When 50% plants were at the pre-flowering stage (pre-emission of racemes, when it was possible to see the racemes), approximately 3 months after transplantation to a greenhouse, plants were transferred to pots with 10 kg non-sterile soil. One week later, the numbers of tillers, racemes per inflorescence, spikelets per raceme and the raceme length in each inflorescence were evaluated, and the evaluations were repeated every 7 days in all plants. The percentage of flowered tillers per pot in each count was evaluated by counting all tillers and marking those that had flowered. In each inflorescence, racemes and the number of spikelets per raceme were counted, and the length of all racemes was measured with a ruler (cm). In order to prevent seed loss, racemes of each plant were wrapped in narrow mesh fabric and cut together with the fabric after all seeds had fallen. After collection of all seeds from each pot (13 months after transplantation), seeds were weighed to determine the weight of seeds and later placed in a seed blower to determine the number of seeds per gram and the number of pure viable seeds. Two subsamples of 100 seeds were soaked in water for 12 hours. A longitudinal section was made through the embryo, and seeds were placed in a 0.5% tetrazolium solution at 30°C for 4 hours. Seeds were evaluated according to the acquired color of each seed, and classified as viable or not viable (Brasil, 1992). Germination test was performed on all pure seeds. The germination test was conducted with four subsamples of 100 seeds, sown on two leaves of filter paper, moistened with 2.5 times its weight of distilled water, in transparent gerbox plastic boxes, which were kept in a germinator at an alternating temperature of 15-5°C (15°C for 16 hours and 35°C for eight hours), in the presence of light for eight hours. Twenty-one days after the start of the germination test, seedlings considered normal (with well-developed radicle and coleoptile) were counted and germination percentage was calculated (Brasil, 1992).

Experiment 2: Resistance of seeds to ergot

As ergot outbreaks are difficult to predict, it was necessary to simulate the environmental conditions that enable its development: high relative air humidity (90% minimum), frequent irrigation and temperatures ranging from 20 to 30°C during flowering (Thakur, King, & Rao, 1989). When the main tiller of at least 50% plants of each genotype presented four fully expanded leaves, pots were transferred to a growth chamber for flowering induction with a photoperiod of 12 hours and temperatures of 22°C during the day and 20°C overnight. Inside the growth chamber, pots were placed in plastic chambers to keep the relative air humidity around 90%, and water was sprayed whenever necessary. The first inoculation of the fungus was done at the time the plants were placed in the growth chamber. The following inoculations were performed twice a week, up to 15 days after the finding that 100% racemes had flowered, which occurred 2 months after transfer to the chamber. For inoculum preparation, 50 g Marandu Palisade grass panicles with ergot symptoms (honeydew) from a naturally infested commercial seed production field were used. Infected seeds were shaken for 15 minutes in 500 mL distilled water. The suspension was sieved through a 325-mesh sieve, and its concentration adjusted to 1×10^6 conidia mL^{-1} in a Neubauer chamber (haemocytometer). For inoculation of panicles with the conidial suspension, 5 mL solution was sprayed per panicle. Each panicle was wrapped in narrow mesh fabric to prevent the seeds from falling. Panicles were harvested at the beginning of the maturity stage and seed evaluations were carried out at the Laboratory of Mycology and Plant Pathology of the Biological Institute. Collections were carried out over 8 months and finished after flowering and collection of all inflorescences of the genotypes. In order to evaluate the occurrence of ergot, seeds were directly inspected under a stereomicroscope (Neegaard, 1979) and the results were presented in incidence (%) of the fungus in seeds. The appearance of sclerotia in seeds and ergot formation (characterized by seed clusters presenting this symptom) were verified. In the case of a seed cluster with the ergot symptomatology, seeds were evaluated on slides analyzed under an optical microscope to confirm the presence of *Sphacelia* spp., which corresponds to the imperfect or anamorphic form of *Claviceps* spp. The number of panicles produced per pot and the number of seeds per pot were counted and classified into apparently healthy or infected (those that had sclerotia or were infected with *Sphacelia* spp).

Results and discussion

Experiment 1: Seed production potential

All Brachiaria hybrids flowered throughout the 13 months of the experiment (Table 1), contrasting with the absence of flowering in Marandu Palisade grass. Photoperiodic requirements for flowering of some Brachiaria grasses, among them Marandu Palisade grass, have not yet been conclusively elucidated (Souza, 2001). Probably, the photoperiod was too short for floral induction (Souza, 2001). Thus, it was not possible to compare the hybrids to Marandu Palisade grass.

Table 1. Morphological characteristics, seed production potential and infected tillers and seeds of Brachiaria grass hybrids and the Marandu Palisade grass cultivar.

	BH1619	BH1810	Mavuno	BH1516	Marandu	CV(%)
	Morphological characteristics					
Tillers (number per pot^{-1})	198 ab	243 a	178 b	218 ab	-	13.6
Flowering tillers (%)	59 a	58 a	62 a	55 a	-	10.5
Apparent seed weight (g pot^{-1})	14.6 a	4.5 b	7.5 b	16.7 a	-	12.27
Pure seed (g pot^{-1})	0.8 b	0.4 b	1.7 b	4.9 a	-	31.36
Racemes (number per $tiller^{-1}$)	4 a	3 a	3 a	4 a	-	18.8
Raceme lenght (cm)	7.9 a	7.0 ab	8.4 a	6.0 b	-	11.63
Spikelets (number per $raceme^{-1}$)	26 b	28 ab	34 a	28 ab	-	13.5
Seeds (n° g^{-1})	172 a	114 bc	102 c	120 b	-	1.6
Germination (%)	16 c	29 b	63 a	51 a	-	6.1
	Infection characteristics					
Tillers (number per pot^{-1})	10 b	9 b	17 ab	28 a	4 c	13.9
Infected tillers (number per pot^{-1})	7ab	8ab	14 a	13 a	3 b	18.6
Infected tillers (%)	70 a	83 a	88 a	43 b	95 a	16.9
Seeds (number per pot^{-1})	364 bc	454 b	970 a	890 a	186 c	6.9
Infected seeds (number per pot^{-1})	52 a	135 a	132 a	57 a	74 a	16.4
Infected seeds (%)	15 ab	28 ab	15 ab	8 b	38 a	24.2

CV: coefficient of variation. Different letters, in the same row, indicate significant differences between treatments ($p < 0.05$).

For the BH1810 hybrid, a higher number of tillers per pot were found, while for Mavuno Palisade grass, the lowest number of tillers was observed. However, the percentage of flowered tillers was similar between hybrids, ranging from 55 to 62%. One of the main determinants of forage seed productivity is the number of reproductive tillers (Carámbula, 1981), which can be highly influenced by management practices within each species, such as the number, season and cutting heights, time of application and amount of nitrogen fertilizer, as well as the date of planting (Souza, 2001). In the present study, all genotypes were evaluated under free growth and received the same dose of nitrogen. In this context, differences in the percentage of reproductive tillers were due to differences between genotypes or to different nitrogen fertilization requirements between them. There was no difference in the mean number of racemes per tiller between hybrids, despite a 25% variation between BH1810 and Mavuno Palisade grass (3 racemes tiller^{-1}) and between BH1619 and BH1516 (4 racemes tiller^{-1}). The Mavuno Palisade grass hybrid has longer racemes, while BH1516 has shorter racemes. The Mavuno Palisade grass hybrid presented the highest number of spikelets per raceme, while the lowest value was recorded for BH1619. Thus, there seems to be a trade-off mechanism between the number of racemes in BH1810 and Mavuno Palisade grass and size of the racemes, since for both genotypes, a smaller number of racemes resulted in longer racemes, and a greater number of spikelets per raceme. The opposite pattern was observed for BH1516: shorter racemes resulted in a higher number of racemes tiller-1.

There was a difference between the hybrids regarding apparent seed weight and the weight of viable pure seeds per pot, with the highest apparent seed weight for BH1619 and BH1516. The BH1516 hybrid produced a greater weight of viable pure seeds than the other evaluated hybrids. BH1619, despite the high apparent seed weight, produced one of the lowest values of viable pure seed weight. In the apomictic reproduction in most Brachiaria grasses, the process of meiosis during gamete formation frequently presents abnormalities, resulting in a large number of infertile florets (Reusch, 1961). Stur and Humphreys (1987) found that, on average, only 22% spikelets of *Brachiaria decumbens* produced seeds, even in an area under specific management for seed production. Observations made by Souza (2001) with Marandu Palisadegrass revealed that even in well-fertilized areas, the percentage of seeds formed is up to 35%. Thus, it is possible to infer that the percentage values of viable pure seeds in relation to the apparent seed weight observed in Mavuno Palisade grass (23%) and BH1516 (29%) are acceptable. Adamowski, Pagliarini, and Do Valle (2008), evaluating the meiotic behavior of three hybrids of *B. ruziziensis* x *B. brizantha*, reported that all hybrids presented a high frequency of abnormalities in meiosis and that this could seriously compromise seed production. This may have been the case for the BH1619 and BH1810 genotypes, which had only 6 and 8% viable pure seeds, very low values when compared to those previously described for *B. decumbens* and *B. brizantha*, the Mavuno Palisade grass and BH1516 genotypes. As Marandu Palisade grass did not flower in the present experiment, it was not possible to use it as a control for this comparison.

Brachiaria hybrids differ in number of pure seeds per gram, with BH1619 producing lighter seeds and Mavuno Palisade grass heavier seeds. The germination percentage of the Brachiaria grass hybrids varied from 16 (BH1619) to 63% (Mavuno Palisade grass), and Mavuno Palisade grass and BH1516 showed the highest germination rates, one of the high physiological seed quality indication (Souza, Peres, Coutinho Filho & Justo, 2015). According to Carámbula (1981), larger seeds usually develop into stronger and more vigorous seedlings, and apparently, this is a difficult problem to solve by means of plant breeding methods. In this sense, Wutoh, Hutton, and Pritchard (1968) stated that higher individual seed weight may be related to higher seedling germination. This was corroborated in the present study, since a higher number of seeds per gram with a lower percentage of germination was verified for BH1619, and a low number of seeds per gram and higher percentage of germination for Mavuno Palisade grass, showing a negative correlation (r2 = -0.55, p = 0.0006, Spearman) between the number of seeds g-1 and germination. Wutoh et al. (1968) and Souza et al. (2015) also observed that greater individual seed weight can be advantageous, since it can lead to faster initial growth and better adaptation to the environment. According to Simeão, Valle, and Resende (2017), seed yield has rarely been considered a selection criterion in the early stages of breeding programs, but it is an essential attribute for a commercial forage grass genotype to be competitive in the market (Fang, Aamlid, Jørgensen, & Rognli, 2004).

Experiment 2: Resistance of seeds to ergot

The first visual symptoms of ergot, 'honeydew', were visually observed 35 days after the first inoculation in all Brachiaria grasses. The fungus that causes ergot (*Claviceps* spp.) infects the unfertilized ovary of plants and replaces the seeds with an elongated black fungal body (sclerotia), which is a protective structure (Mitchell & Cooke, 1968 ; Uppala, Wu, & Alderman, 2016). Under suitable temperature and rainfall, sclerotia germinate and produce fruiting bodies (capitula), which release airborne ascospores that serve as a primary inoculum (Alderman, 1993). Host infection occurs when an ascospore germinates in a plant floret and grows profusely into the ovary (Perez, Gundel, Marrero, Arzac & Omacini, 2017). The pathogen produces 'honeydew', a droplet of sweet plant sap containing fungal conidia, which can be dispersed by many agents, such as wind, water or insects visiting flowers, and cause new infections. The result of the infection process is the replacement of the seed with fungal biomass that eventually forms a sclerotium.

The number of flowered tillers per pot ranged from 4 to 28 (Marandu Palisade grass and BH1516, respectively). Although the BH1516 and Mavuno Palisade grass hybrids did not differ in relation to the total number of flowered tillers, a higher number of healthy tillers and a lower percentage of infected tillers were observed for BH1516. There was no difference in the percentage of infected flowered tillers between BH1619, BH1810, Mavuno Palisade grass and Marandu Palisade grass (Table 1).

The Mavuno Palisade grass and BH1516 hybrids produced more seeds than the other hybrids, as well as a higher number of asymptomatic seeds (apparently healthy seeds). For the Marandu Palisade grass, a lower number of seeds were produced with a higher percentage of infected seeds, while for the BH1516 genotype, a high number of seeds were produced with a lower percentage of infected seeds. Thus, the greater the infection, the less the number of seeds produced. Therefore, we can infer that there seems to be a relationship between the presence of the fungus or disease in plants and the lower production of seeds. Similar behavior was described by Gomes, Rocha, Pereira, and Souza (2017), who evaluated the quality of wheat seeds subjected to inoculation with the fungus *Pyricularia oryzae*. Marchiet et al. (2008) analyzed the ergot progress and control in *B. brizantha* seeds and reported a panicle infection rate of 63% for the Marandu Palisadegrass, 37% lower than that found in this study, in which 95% flowered tillers were infected. These authors also observed that only 20% seeds were infected, against the 38% of infected seeds verified herein. However, these studies were performed under field conditions, while the present study was carried out under controlled environment conditions. Vechiato, Aparecido, and Fernandes (2010) investigated the occurrence of *Claviceps* spp. in commercial samples of Brachiaria grasses by dry seed inspection, reported a high frequency of the fungus in *B. brizantha* seeds over 2 years, being 74.6% in Marandu Palisade grass and 89.7% in Xaraés Palisadegrass. According to these authors, in *B. decumbens* cv. Basilisk the frequency was 17.8%, which suggest high susceptibility of *B. brizantha* to the fungus. The Brachiaria grasses most susceptible to infection with ergot were Marandu Palisade grass and BH1810. On the other hand, Mavuno Palisade grass and BH1619 appear to be moderately resistant to this disease, whereas BH1516 proved to be an interesting alternative for use in areas with a history of ergot infection.

Conclusion

The Mavuno Palisade grass and BH1516 hybrids have similar potential for seed production and, therefore, commercial cultivation. In addition, the BH1516 hybrid was resistant to ergot, making it a better choice for use in infected areas. It is essential to consider seed production potential and ergot tolerance as criteria for selection of Brachiaria grass for use in infected areas utilized for seed or forage production. The flowering behavior of the Marandu Palisade grass is probably controlled by the photoperiod, as it flowered in the growth chamber under constant light and temperature conditions and did not flower in the greenhouse, where the light period varied according to the season. Grass-breeding programs should consider the improvement of seed traits, especially seed size and yield, and Ergot resistance, important traits for pasture establishment and seed commercialization.

Acnowledgements

We are grateful to Ph.D. Olga Maria Ripinskas Russomanno (*in memorian*), scientific researcher of Biological Institute, for providing technical assistance regarding *Claviceps* analysis. Thanks are extended to *Fundação de Apoio à Pesquisa Agrícola* for providing legal and financial support with the public-private partnership.

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