MANAGEMENT OF THE FUNGUS COMPLEX CAUSED BY ERGOT IN *Brachiaria* SPECIES

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Introduction

Brachiaria is currently a large genus that contains about 100 species distributed in all tropics, mainly in Africa. The habitats where these species grow are very varied, most of them located in the savannas. The agronomic interest generated by this genus focuses on several species that are used to develop pasture (Miles et al., 1998). In addition, its adaptability to poor and acidic soils has enabled a rapid intercontinental distribution. Due to this characteristic, diseases have also increased dramatically and have become a limitation of increased importance for their production. The spread of most of the species belonging to this genus is done by using seeds, being these the most effective system for the spread of pathogens (García and Pineda, 2000).

Brachiaria species are exposed to various diseases caused by fungi and viruses preventing the normal development of the plants. Most of these diseases occur in Africa, *Brachiaria's* diversity center; however, in recent years, diseases have acquired importance also in tropical America, where the *Brachiaria* species have been introduced and are widely cultivated (Miles et al., 1998). Among the diseases that cause major problems in the seed production process is the Ergot disease, which is caused by *Claviceps* species.

Establishment of plots for seed production of the genus *Brachiaria*

Currently, CIAT genebank has an *ex situ* collection of *Brachiaria* which has 595 accessions represented by 20 species. This collection is located in Santa Rosa, Popayán, Department of Cauca, Colombia (1,750 m.a.s.l, latitude 2°.31' N, length 76°.38' O, area 5,555 m^2); the station has inceptisol soils, low in phosphorus (under 5 ppm), pH 5.5 and high organic matter (20-30%).

To set lots or plots of seed production of the genus *Brachiaria* for genebanks, one must identify areas that have good drainage in which no material of the genus was previously planted, and thus preventing contamination of the accessions that will be set there for the multiplication process.

It is recommended to take into account certain criteria of sowing in order to take advantage of the available space and thus optimizing the processes of maintenance and harvesting of the plots. For this reason, the plots must be sown in rows to facilitate the access during the processes of harvesting, fertilizer application, application of fungicides, maintenance works, among other activities; avoiding that the staff in charge may cause mechanical damage (treading, panicles break) to the planting material.

If the planting is done through the use of sexual seed, seeds can be planted in two rows, furrows or parallel rows of 10 m long, keeping a distance of at least 50 cm between rows; planting approximately 500 seeds per row. In addition, when setting several plots in field these must have approximately a separation distance of 5 m as shown in Figure 1.

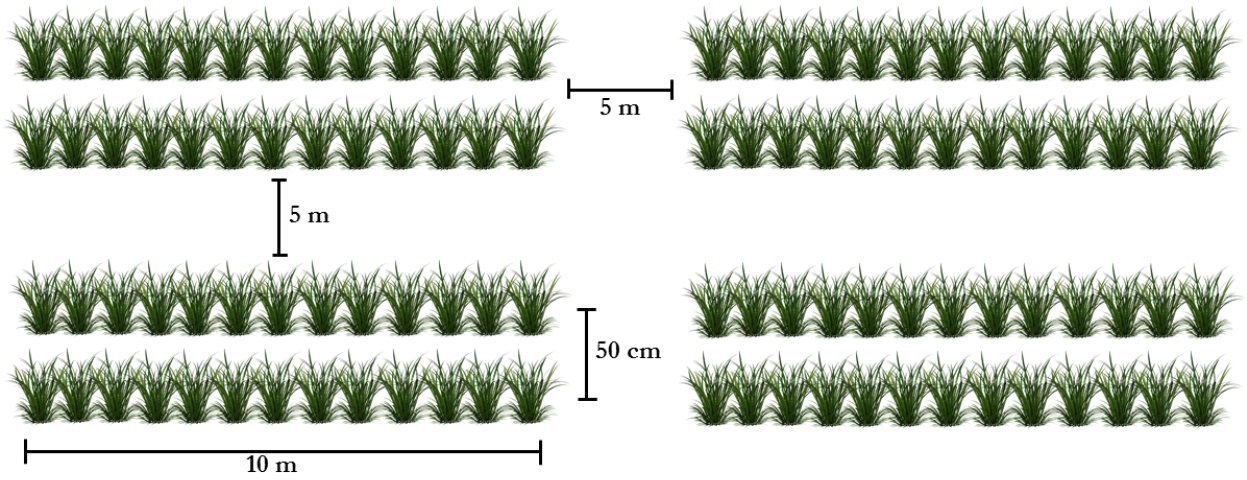

Figure 1. Establishment of plots using sexual seed.

If planting is done by vegetative material (stolons or tillers) one should be aware that the tillers to be planted must have a minimum distance of 50 cm between them as shown in Figure 2, also keeping the 10 m long and 5 m of distance between plots.

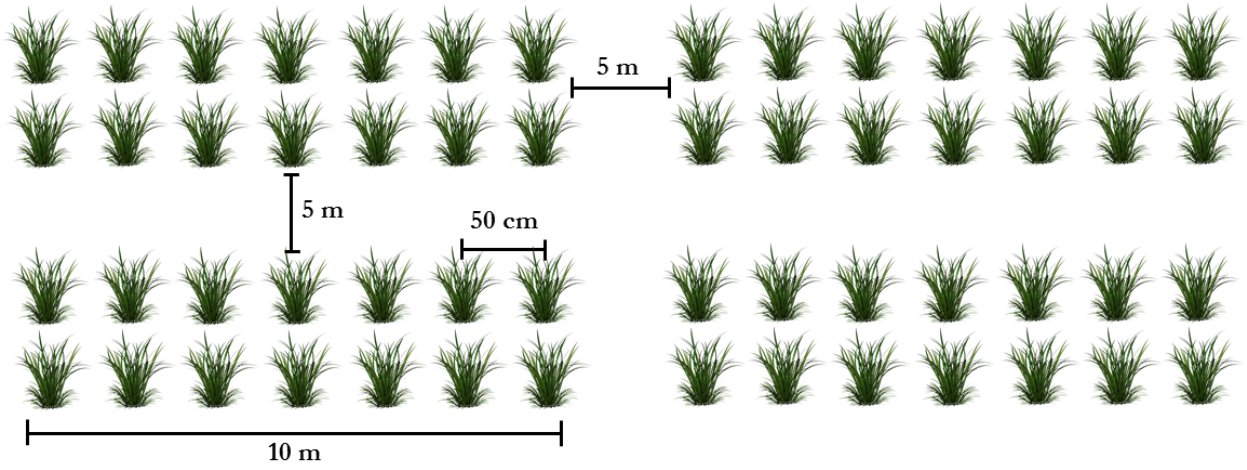

Figure 2. Establishment of plots using stolons or tillers

The planting distances between accessions or between plots should be 5 m approximately. It should be noted that this distance is used for the majority of the species since there are materials with aggressive growth, generating large amount of foliage above the meter in height. If the material presents these characteristics it is recommended to extend the distance between plots.

It is important to highlight that there are some species belonging to the genus *Brachiaria* possessing a greater adaptability to marshy lands or with lots of water, but unfortunately they are materials that have very little flowering and empty seed (absence of the seed filling), for this reason they must be established in geographical areas other than those observed in Popayán, and must be multiplied by vegetative means.

Ergot disease

This disease is caused by some *Claviceps* species; it has been registered in *Brachiaria* in Africa - Ethiopia, Kenya, Malawi and Zimbabwe - in Australia and India. In South America, this fungus has been reported in Brazil and Colombia. It is known that the mature sclerotia of *Claviceps* spp. produce toxic alkaloids, which can cause serious injury or death to cattle and humans when consumed in great quantity (Miles et al., 1998).

The *Claviceps* genus corresponds to a group of fungi belonging to the Ascomycetes class, Hymenoascomycetidae subclass, order Xylariales, Clavicipetaceae family and their representative species is *Claviceps purpurea* (Fr.) Tul, causal agent of ergot of rye (Alexopoulos & Mims, 1979). *Claviceps* spp. and its asexual or imperfect condition (*Sphacelia* sp.) parasitize more than 600 species of monocotyledon plants of the families Poaceae, Juncaceae and Cyperaceae (Kren, 1999; Lenné, 1994). About 45 species of *Claviceps* have been described, but presumably many species can exist only in their asexual stage and therefore have not been reported (Pažoutová, 2003).

Cycle of the disease

The process begins when the conidia of the pathogen germinate through tubes that penetrate the stigma, which grow downwards by the style and colonize the ovary. The ovary is converted into a fungal mass (stroma) that is expelled through the sugary exudate, which contains macroconidia and microconidia (Bandyopadhyay et al., 1998) (Fig. 3).

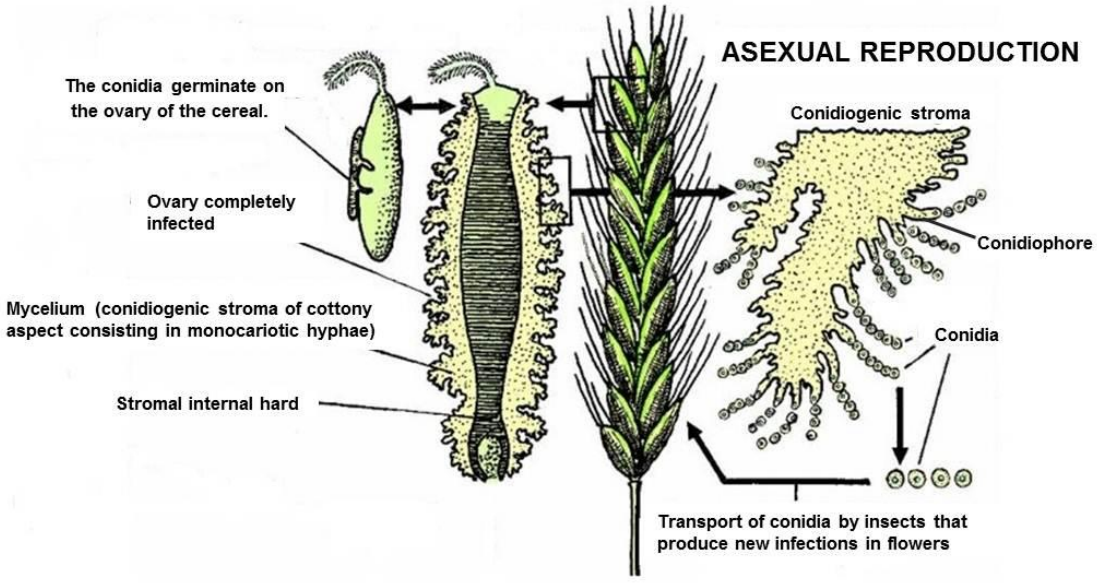

Figure 3. Representation of the conidial state of Ergot[1].

[1] Paul L. Schiff, Jr., PhD. Ergot and its Alkaloids. School of Pharmacy, University of Pittsburgh. Available at: http://www.summagallicana.it/lessico/s/segale.htm. Accessed on 10 July, 2014.

Later, under appropriate environmental conditions, the stroma is transformed into a resistant sclerotia. The teleomorph of *Claviceps* is formed when the sclerotia germinates producing a stroma with globose stem which contains a perithecium and ascospores (Fig. 4). So far, the sclerotic stage has not been observed in Colombia (Miles et al., 1998). Since the perithecium does not germinate easily under natural conditions, the role of ascospores in the Ergot's epidemiology is unknown (Bandyopadhyay et al., 1998).

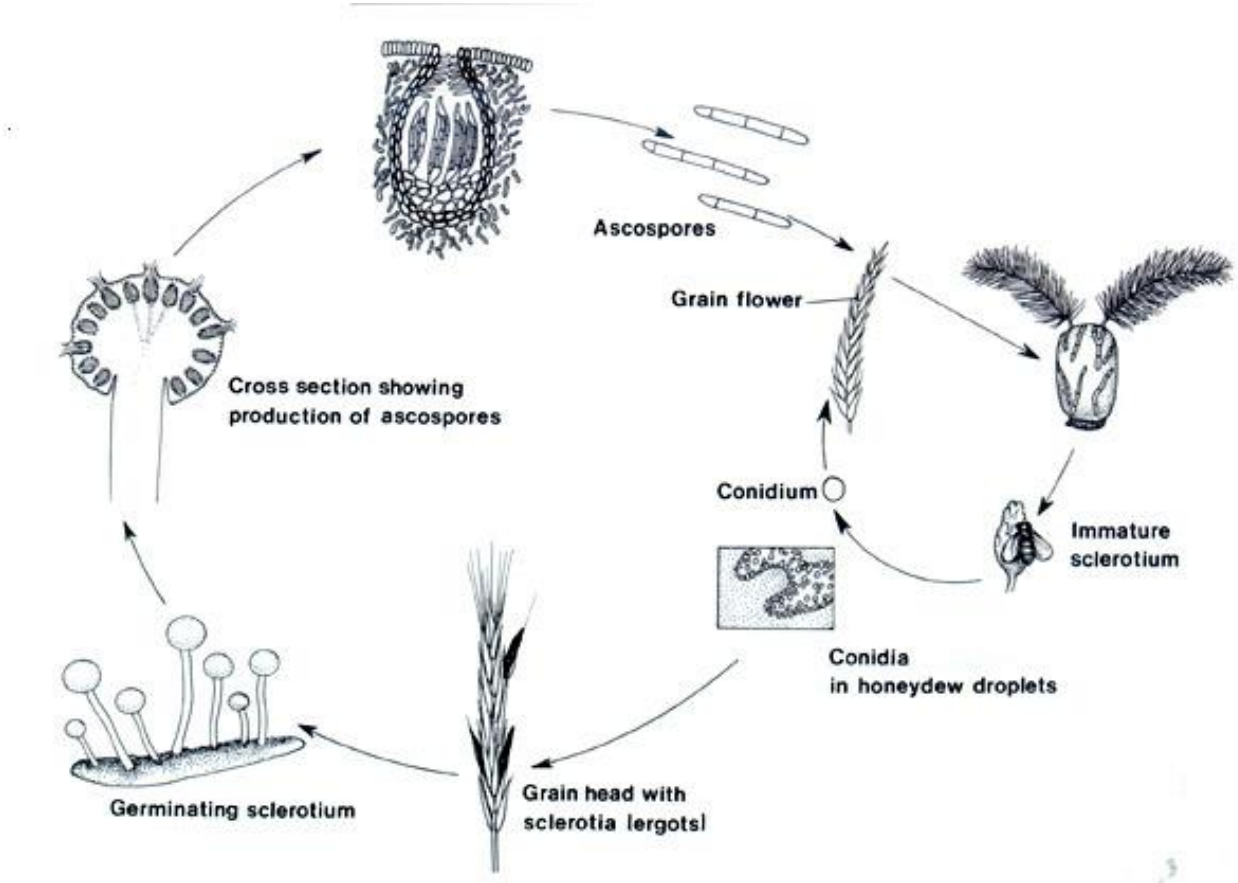

Figure 4. Complete cycle complete of the Ergot disease[2].

When the flowers open, the stigmas emerge and their ovaries become receptive, being able to be pollinated and fertilized in a few hours. The stigma of a grass flower is large and feathery; this characteristic facilitates the capture of pollen as well as conidia that are transported by the wind (Fig. 5). The conidia are the primary inoculum and manage to germinate and infect the ovary (Schumann, 2000). The process of germination of conidia takes approximately two to three days to colonize the whole ovary.

[2] Cynthia Bertelsen. Mushroom: A Global History. Available at: http://gherkinstomatoes.com/2011/02/03/st-anthonys-fire-the-story-of-ergot-du-seigle-ergotism/. Accessed on 6 August, 2014.

Figure 5. Flowering stage of *Brachiaria* susceptible to the colonization of *Sphacelia*.

When the ovary is fertilized, the flower resists the infection of the fungus; this way one can determine that susceptibility occurs when the stigmas are receptive and not after their ovaries have been fertilized.

Five days later, the conidiogenous stroma develops onto the surface of the infected ovary which produces great amount of conidia (Fig. 6). These conidias are exuded with a sweet, viscous nectar, and light brown to toasting color (Fig. 7). The produced conidia are the secondary inoculum and are dispersed to other flowers through physical contact, splashing rain and insects. This exudate attracts insects to the anemogamus flowers. Contaminated insects with conidia can visit healthy flowers, where new infections begin (Schumann, 2000).

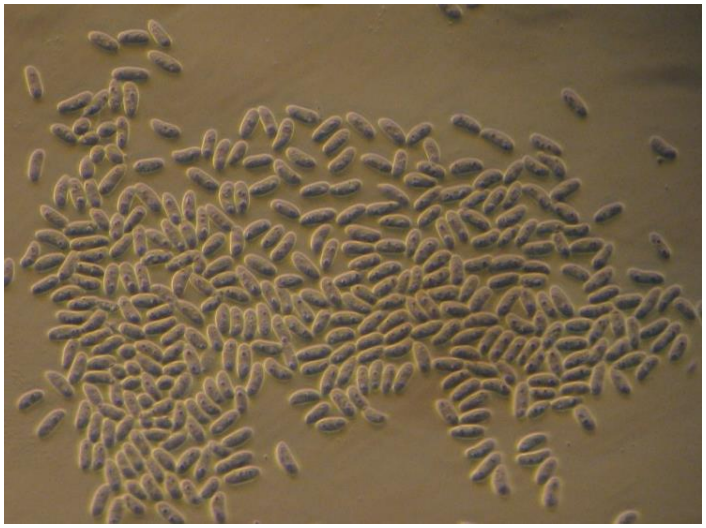

Figure 6. Microphotography of *Sphacelia* sp. conidia obtained from a drop of sweetened exudate.

Figure 7. Sweetened exudate characteristic symptom caused by the presence of *Sphacelia* spp.

The sugary exudate attracts insects and encourages the growth of other fungi such as *Fusarium heterosporium*, *Cerebella* spp, *Alternaria* spp., *Aspergillus* spp., *Curvularia* spp, *Chaetomium* spp., *Drechslera* spp, *Cladosporium* spp, *Nigrospora* spp, *Penicillium* spp, *Phoma* spp, among others (Fig. 8) (Medina, 2009).

Figure 8. Colonization of saprophytes and quarantine fungi on the sweetened exudate.

Figure 9 presents a diagram of the different observed phenotypic states of the genus *Brachiaria* in the seed production process. Similarly, different symptoms caused by Ergot in each stage of the infection are observed.

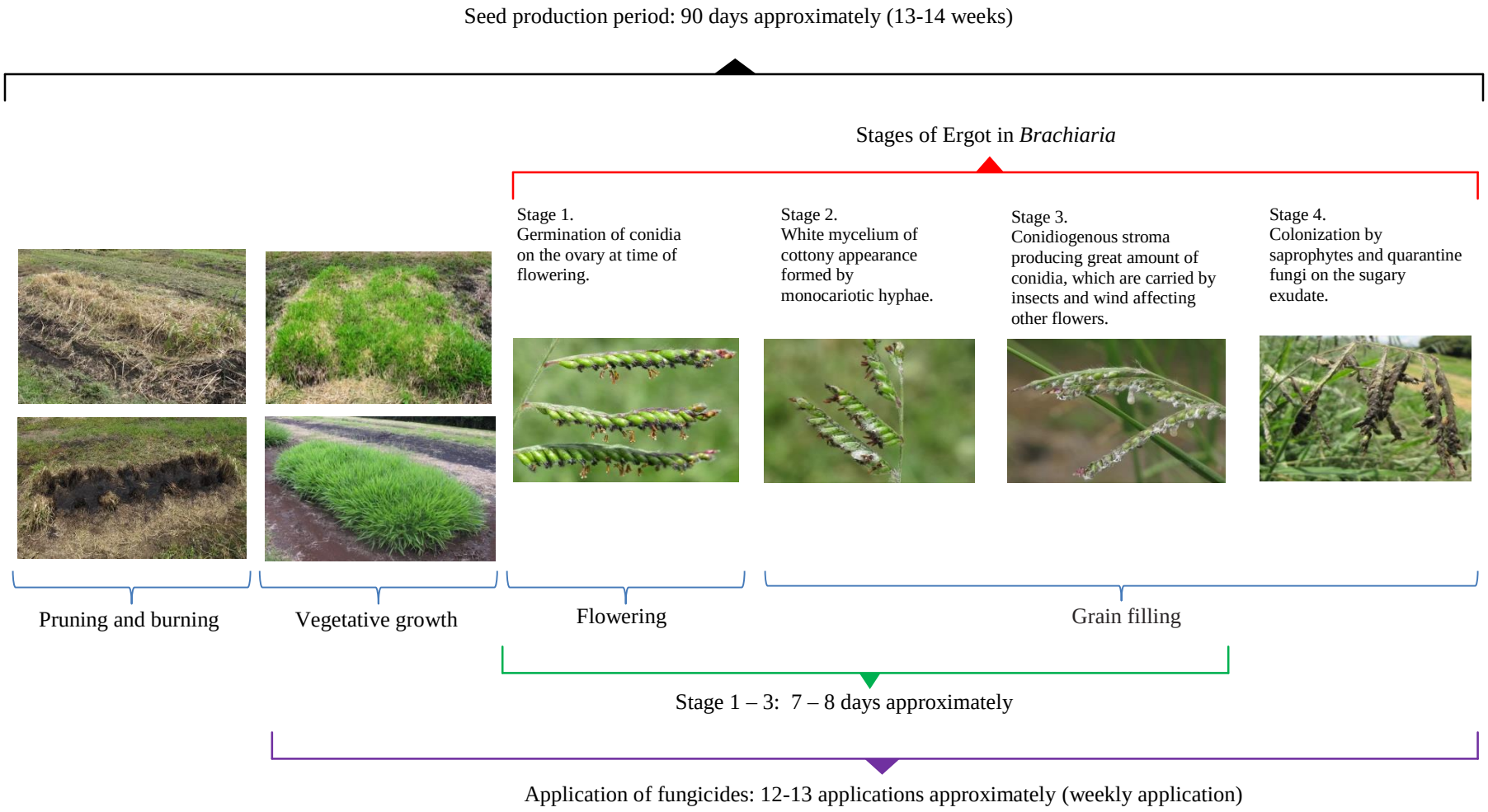

Figure 9. The disease cycle of Ergot in phenotypic stages of *Brachiaria* spp. (CIAT experimental station - Popayán).

Pre-Harvest Process

In order to find effective methods for the management of Ergot and the fungal complex associated with this disease several investigations have been done. Among these are *in vitro* assays of biological control with antagonistic bacteria against quarantine fungus such as *Drechslera* sp., *Phoma* sp. and *Curvularia* sp. (Balcázar et al., 2003), and the use of extract of seeds of *Clitoria ternatea* (Kelemu et al., 2005) (Medina, 2009), without satisfactory results for the control of this disease.

Field tests were performed with accessions of *Brachiaria* from CIAT genebank using Propiconazole and Tebuconazole+Trifloxystrobin with the purpose of evaluating its effectiveness in the inhibition of fungal growth of *Sphacelia* sp. and through this way to find a mechanism of controlling the Ergot disease. Analyses of seed health were performed on seeds obtained after using the above-mentioned treatments, and a decrease in the forming units of spores (Forming Unit of Colonies-FUC) of quarantine fungus was observed when these were compared against control plants (Medina, 2009).

Also, the Germplasm Health Laboratory (GHL) conducted field trials in Popayán using biological products based on *Trichoderma* spp. for the control of this complex fungus, without obtaining satisfactory results.

Field evaluations determined that there is a decrease in the incidence of the Ergot on inflorescences of *Brachiaria* spp. in the localities of Santander de Quilichao and Palmira compared to Popayán, but flowering, seed production and filling of seed is much greater in this last locality; for this reason, experimental trials in *Brachiaria* have been conducted in this experimental station (Medina, 2009).

Based on these results for the control of pathogens fungus affecting the seeds of genus *Brachiaria* production, we begin to use two commercials fungicides based on Propiconazole and Tebuconazole+Trifloxystrobin, respectively, in one dose of 3 ml per liter of water of each, making one application every eight days alternating these products weekly (Fig. 10A).

These two fungicides work systemically, i.e., they are absorbed through the foliage or roots and move throughout the whole plant. Systemic fungicides affect various stages of the life of the fungus.

In addition to these two products, we apply Azoxistrobina+Difeconazol with 2 ml/liter of water and copper hydroxide with 100 gr dissolved in 18 liters of water every fifteen days, in order to control quarantine fungal diseases (Fig. 10B). The Azoxistrobina+Difeconazol is a systemic fungicide and of contact, of natural origin, with broad spectrum of control. It has "triple action," with preventive, curative and antisporulant activity. Its movement is via xylem, fully protecting the leaves and new sprouts.

A)  B) 

Figure 10. A) Application of fungicides as spray; B) Application of fungicides as solution.

The contact fungicides like copper hydroxide, despite its name, do not have to touch the fungus to kill him. Its function is to establish a protective film over the plant surface that prevent the germination of the spores or the development of the initial mycelium before infection occurs.

Harvest process

The harvest process begins approximately 90 days after having been established the plot, i.e., the panicles are loaded with seeds with the degree of maturation suitable for its harvesting (Fig. 11).

 

Figure 11. Phenotypes of mature *Brachiaria* ready for the harvest process.

Materials:

- ✓ Plastic buckets.
- ✓ Wide mouthed plastic container.
- ✓ Detergent powder.
- ✓ Sodium hypochlorite.
- ✓ Towel or cotton cloth.
- ✓ Paper bags.

- ✓ Muslin bags.

The process starts with the washing of hands of the person responsible for performing the work of harvesting, using water and detergent powder, and then drying them using a towel or cotton cloth completely clean. This same process of washing must be done with the plastic tray in which the harvested seed will be collected. Cleaning of hands and plastic trays must be repeated between plot and plot (Fig. 12).

A)  B) 

Figure 12. A) Materials used in the harvest; B) Cleaning of materials used in the harvest process of *Brachiaria*.

All implements used in the harvest must be previously disinfected with water, detergent powder and 5 % sodium hypochlorite (buckets, plastic trays, towels and muslin bags).

In the past, this culture of cleaning was not used, and a person in charge of the harvest could have been vector of diseases by touching infected plants from one plot to another.

When the plots show plants mature enough for harvesting, the seeds are obtained manually or gently rubbing the inflorescences so only the ripe seeds are shed, repeating the process several times; method known as milking. During the process the harvested seeds are falling within the plastic tray (Fig. 13).

 

Figure 13. Harvest process using the milking method.

Since the processes described above have been implemented, the symptomatology caused by Ergot did not show up. However, it is important to highlight that a selection of inflorescences to be milked should be done, and those that show presence of fungi or sugary exudate should be avoided. Only spikes visibly healthy should be harvested.

The selection of spikes free of fungal symptoms is critical in the harvest; therefore, all contaminations and mixtures of healthy seeds with infected seeds should be avoided to the maximum.

The seeds obtained in the harvest process are saved in new paper bags to avoid the mixing of materials. Before being located in paper bags, a cleaning of the harvested material is done by removing any dirt or plant material other than seeds (Fig. 14).

Figure 14. Cleaning and packaging of seeds collected in the harvest process.

The bags are labelled with date, code of the accession, origin and location of the plot and number of cutting[3]. Paper bags with harvested seed are sealed with metal staples and placed in bags of clean muslin which is transported by the person in charge of the harvest throughout the whole process (Fig. 15). Once the travel to harvest the plots is completed, the muslin bags are hung to air for subsequent transport to the drying devices on the premises of the Genetic Resources Program.

Figure 15. Types of packaging used in the harvest process.

[3] A phytosanitary analysis is done in the genebank for each crop or cut done to the plot in order to take control of pathogens and thus take appropriate measures for its management.

In previous years, the harvested seeds were placed in muslin bags intended for this purpose, and these were put on the ground. This culture has been abolished completely, reducing the direct contact of seeds with saprophytes and quarantine fungi that may be present in the soil.

Post-Harvest Process

Once the process of harvesting is finished, a pruning of the tillers is carried out in each of the plots at 20 cm above the floor, this in order to stimulate the rapid growth and development of the foliage, as well as the proliferation of inflorescences (Fig. 16A). A continuous dense vegetative state of the pasture should be avoided since it inhibits flowering. The application of fertilizers at ground level should be in addition to the pruning process.

Once the process of pruning of the tillers is finished, the resulting plant material is located on the plot (Fig. 16B). This is done in order to prevent contamination of neighboring plots and to accumulate enough material that will serve for the burning process after a drying period. This process of locating the trimmed material on the plot is done only during the summer, since the heat produced by the sun rays helps to the process of drying. In rainy season the trimmed material is removed from the place to disposal sites since if it were placed on the plot the high humidity would produce adverse effects on the tillers as rot and proliferation of saprophytes and quarantine fungi. Special care should be taken when transporting material to the disposal sites since it contains waste (seeds, stems, etc.) that could contaminate the neighboring plots.

A)  B) 

Figure 16. Pruning process after the harvest.

Once the plant material resulting from pruning is sufficiently dry, a burning of the plot is performed in order to reduce the inoculum source of quarantine pathogens that may be present in soil, as well as in the seeds fallen during the process of the harvest, and also to prevent contamination of neighboring plots (Fig. 17A)[4]. Similarly, the process of incineration of the plot stimulates the vegetative development by providing large amount of minerals to the ground as a result of this process (Fig. 17B).

[4] Note: it is important to clarify that not all 17 species respond well in regrowth after burning.

A)  B) 

Figure 17. A) Burned plot; B) Start of the vegetative development of the plot.

Eight days after having done the burning of the plot the process of application of fungicides begins in order to provide protection to the plot against diseases during the vegetative state.

In order to achieve a better effectiveness of the applied products, these are mixed with an adjuvant for agricultural products based on a mixture of ethoxylated alcohols and aryl polyglycol polyethoxyethanol of non-ionic character which has characteristics of surfactants, wetting, penetrating, and adherents, among others. This last feature prevents that the applied products are washed with rain. This adjuvant is used primarily in the rainy season.

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