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Influence of pastoral systems on *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae) and the entomopathogen *Metarhizium anisopliae* (Metsch.) Sorokin

R. M. Pitta¹; S. C. Matiero²; J. de N. Corassa²; F. T. Rampelotti-Ferreira²

¹ Embrapa Agrossilvipastoril

² Universidade Federal de Mato Grosso

Author for correspondence: rafael.pitta@embrapa.br

Abstract: The influence of *Urochloa brizantha* (variety Marandu) grazing systems on *Mahanarva spectabilis* (Distant) and the entomopathogen *Metarhizium anisopliae* (Metsch.) was studied to understand the benefits of integrated systems in pest management. The pastoral systems studied were: (M) monoculture, (SP) silvopastoral and (ICLF). We assessed the number, per square meter, of alive spittlebug nymphs or infected by *M. anisopliae* as well as the demanded number of entomopathogen sprays in each pasture system to control the pest. Throughout the experiment period, *M. spectabilis* was the unique species found. Silvopastoral had a higher number of alive nymphs and a lower percentage of the infected nymphs compared to pasture in monoculture; however, in both systems, only one spray of *M. anisopliae* was enough to keep the pest below its threshold. In agrosilvopastoral system, there was no spittlebugs infestation, consequently, none sprays was demanded. Thus, intensified production systems such ICLF may be more sustainable, considering pest aspects.

Keywords: Spittlebugs; microbial control; integrated systems; agroforestry

Introduction

Brazilian Govern has many strategies to reduce rates of Brazilian greenhouse emissions. Among them, there is the Plan ABC - Low Carbon Emission Agriculture (Brasil, 2012) where production systems like Crop-Livestock-Forest integration (ICLF) are encouraged. This strategy reduces the emission of gases that produce the greenhouse effect. Thus, facilitating more sustainable agricultural methods. These systems are developed to raise both the productivity and financial gain for the rural population and reduce the risks of environmental deterioration, enhancing the chemical, physical and biological soil richness (Santos et al. 2008) as well as communicating scientific know-how on the physiological ecology of the various species of plants and govern their interactions with the flora and fauna of their locale (Santos et al., 2010).

In light of these complex production systems, multidisciplinary studies are required to assess the chances of the success of such method, as well as to technically subsidize growers who decide to adopt those systems to produce food, timber, beef and milk.

Studies in soil nutrient distribution and their effect on the soybean yield (Diel et al. 2014); carbon stocks and soil nitrogen (Sacramento et al. 2013), pasture yield (Paciullo et al., 2011) under shade trees reveal that ICLF bring yield advantages. However, little is known about the influence of these systems on the community of insect pests and their natural.

Pastures host a rich entomofauna community, including several pests (Sousa et al. 2013) such as spittlebugs species (Hernández-Dominguez et al., 2016). *Mahanarva spectabilis* damages the forage growth and production, especially *Urochloa* spp and *Pennisetum* spp. (Fonseca et al., 2016). Both nymphs and adults damage the host plant by sucking sap and injecting toxins which induce plant tissue yellowing and reduce photosynthetic rates (Byers e Wells 1966; Resende et al., 2013; Aguiar et al., 2014). Resende et al., 2013) affirm that eight adults of *M. spectabilis* (Distant) are capable of reducing 60% of *Urochloa ruziziensis* (Germ & Evrard) yield.

Application of the entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) (Hypocreales:

Clavicipitaceae) to control spittlebugs in pasture and sugarcane are usual in Brazil (Hernández-Dominguez et al., 2016). However, the pathogenicity of the fungus depends on abiotic environmental characteristics, including soil moisture, which plays a crucial role in the fungal maintenance and multiplication (Alves et al., 2011).

Some studies have shown that shadow promoted by trees in silvopastoral systems reduce solar radiation, avoid soil moisture loss and increase air humidity (Paciullo et al., 2008; Law et al., 2011; Pezzopane et al., 2015) which contribute to entomopathogen establishment (Meyling et al., 2009).

In this study, we evaluated the possible influence of integrated systems on *M. spectabilis* infestation as well as the efficiency of *M. anisopliae* to control that spittlebug.

Methods

The experiment were carried out from November 2013 to July 2014 in Sinop-MT-Brazil (11° 51'S, 55° 35' W and 384 m altitude), a transition region between Cerrado and Amazon Rainforest (Araújo et al., 2009). The climate of the region is type Aw according to Köppen climate classification, owing a tropical winter and annual average temperature of 25°C, Relative Humidity of 82.5% and a precipitation of 2,550 mm (National Institute of Meteorology 2018).

The three systems evaluated were: (M) monoculture pasture, (SP) silvopastoral system and (ICLF) agrosilvopastoral. The silvopastoral system had triple rows every 30 meters of the hybrid H13 *Eucalyptus*, (*Eucalyptus grandis* vs *Eucalyptus urophylla*), with 3m x 3.5m between the individual trees (Fig.1). Palisade grass (*U. brizantha*) cv *Marandu* was sown between *Eucalyptus* rows at a density of 12 Kg.ha⁻¹ (80% germination). The monoculture pasture was also sown as silvopastoral system. In agrosilvopastoral system, soybean was cultivated between eucalyptus rows during the summer followed by maize in autumn intercropped with palisade grass in order to offer pasture to cattle during the crops off-season (june - october). All production system had 2 ha.

Four plots (4 x 38m) per treatment were installed as perpendicular transects to *Eucalyptus* rows in a way that 16m length faced to south side of the rows (receiving direct sunlight) and 16m faced to north side (under the shade trees) (Figure 1). In the monoculture pasture, four plots of 25m² were randomly arranged and installed within the area.

To establish forage uniformity in silvopastoral systems and monoculture pasture, plants were cut every 28 days to mimic the recommended grazing height of 10 cm (Jayme et al., 2009). All the harvested biomass was transferred away from the site to remove any type of physical barrier that could offer solar radiation protection to spittlebugs nymphs (Chiaradia et al., 2014).

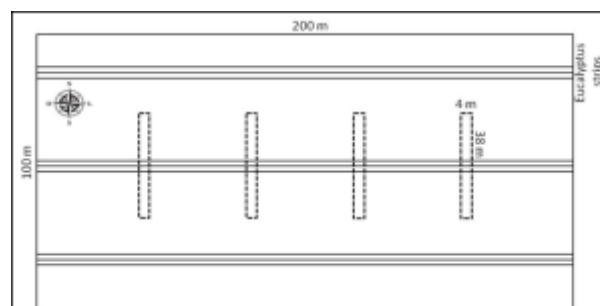


Figure 1 - Silvopastoral and agrosilvopastoral systems layout. Triple lines indicate the *Eucalyptus* rows. Dotted rectangles show the four repetitions of each production system

Quantification of the live and infected nymphs

Every week the evaluations were done on 1m² per sample point. The number of alive *M. spectabilis* nymphs was quantified at the plant base as well as the number of nymphs infected by fungus.

The sampling points in agrosilvopastoral and silvopastoral systems were chosen at 3 and 15m, respectively, from north and south faces of the central *Eucalyptus* hedgerow in a total of four samples per plot. The evaluation ranges were selected prior based on the projection of the tree shade to evaluate the influence of shading on the spittlebug population. In the monoculture pasture, the four samples were randomly selected in each plot.

Samples of adults were collected and sent to Dr Gervásio Silva Carvalho (Pontifica Universidade Católica do Rio Grande do Sul), a specialist in Cercopidae, in order to identify the species occurring in the experimental area.

Control measure

The spittlebugs were controlled whenever each plot reached an infestation of 25 nymphs per m² (Valério & Koller, 1992). Control was performed using a commercial biopesticide of *M. anisopliae* (Metarril®) formulated as wettable powder in a concentration of 2x10⁹ viable conidia. The dosage used was 250 g.ha⁻¹ and a spray volume of 150 L.ha⁻¹.

The number of sprays required in each production system and the total number of insects gathered in each system were used as sustainability parameters to evaluated pastoral system.

Statistical analysis

The data were analyzed about their normality and homoscedasticity in order to decide the use of parametric or nonparametric statistical testing according to the nature of the data sets. All analyses were performed using the R software (R Development Core Team, 2008).

Results and discussion

Quantification of nymphs

M. spectabilis was not detected in agrosilvopastoral system throughout the evaluation period. However, in silvopastoral and monoculture systems, *M. spectabilis* infestation reached the action threshold level (> 25 nymphs per m^2) during the first evaluation on 31/10/2013 (Figure 2) which demanded an *M. anisopliae* spray in both systems. Although the initial *M. spectabilis* infestation in silvopastoral system was almost three fold higher than the infestation in monoculture pasture, a single *M. anisopliae* spray was enough in both systems to maintain the pest population below its threshold throughout the entire rainfall period; this showed that

using *M. anisopliae* as control was effective in both the systems.

In terms of total nymph collected per m^2 during the experiment, a significant difference was observed between the pastoral systems ($p < 0.001$), with the infestation being most prominent in silvopastoral system ($n = 178.44$) than in monoculture ($n = 95.38$) (Figure 3).

In relation to number of nymphs per m^2 in the distances of 3m and 15m from the North and South faces of the *Eucalyptus* hedgerow in the silvopastoral system, there were no different infestations among these distances ($p = 0.94$) (Figure 4).

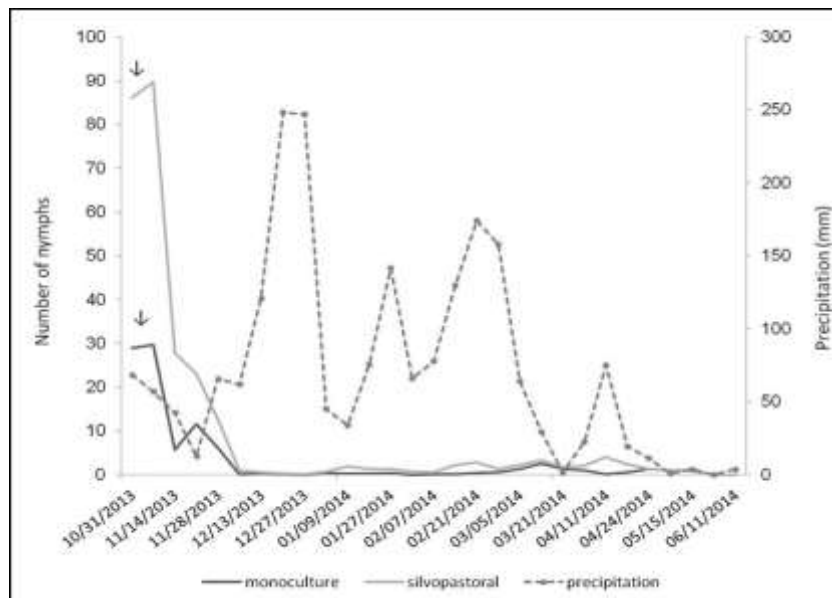


Figure 2 - Population fluctuation of *Mahanarva spectabilis* nymphs in pastoral system and precipitation (mm) during the evaluation period. Arrows indicate when *Metarhizium anisopliae* was sprayed in both systems.

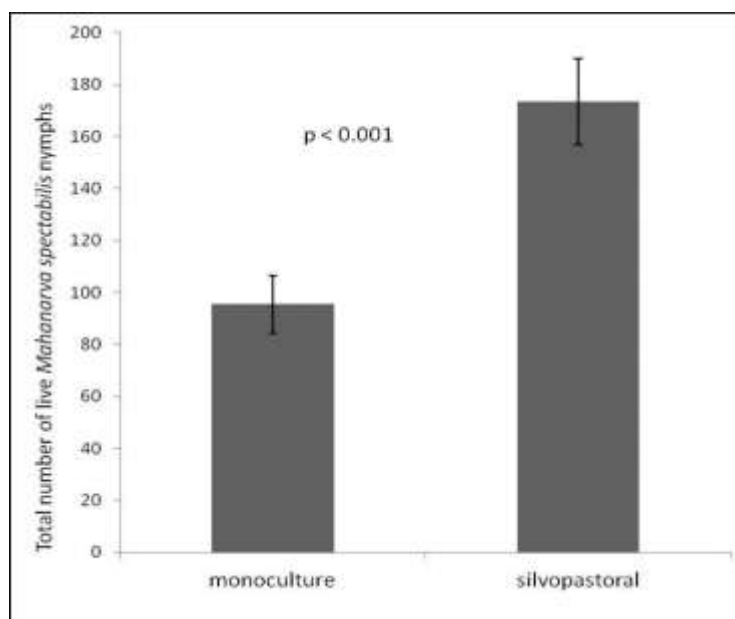


Figure 3 - Total number of alive *Mahanarva spectabilis* nymphs in pastoral systems. Wilcoxon test at 5% probability.

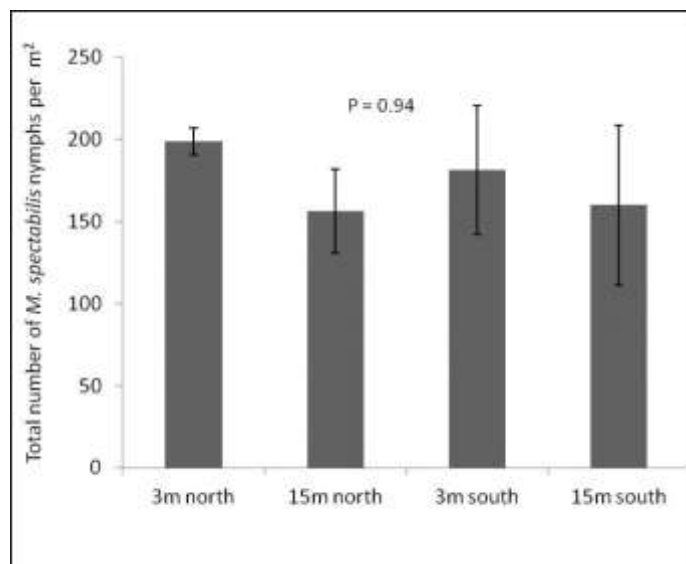


Figure 4 - Total number of alive *Mahanarva spectabilis* nymphs per m² present in the silvopastoral system at distances of 3 m and 15 m on the north side and 15m and 3m on the south side of the central *Eucalyptus* row. ANOVA at 5% probability.

Quantification of infected nymphs

Although the monoculture pasture had the lowest nymph infestation, the percentage of infected nymphs was greater in this system (15.7%) when compared with silvopastoral system (6%) ($p < 0.001$) (Figure 5). Spatial distribution analysis of infected nymphs in silvopastoral system, as well as the number of alive nymphs, show that neither the presence nor absence of *Eucalyptus* shading on pasture has any effect on control efficiency of the *M. anisopliae* on nymphs of *M. spectabilis*, inasmuch as no difference in the number of infected nymphs was

reported between the distances measured (3m and 15m) from the north and south faces of the *Eucalyptus* hedgerow ($p = 0.41$) (Figure 6).

In the pastoral systems with spittlebug infestation (monoculture and silvopastoral), this infestation occurred in the beginning of the rainy season (November 2013) when the total rainfall measured was 239,25 mm, providing enough soil moisture to end the quiescence process of the eggs present in the dry soil (Sujii et al., 2001); thus, rising the first *M. spectabilis* generation as described by Valério (2009) e Auad et al. (2009).

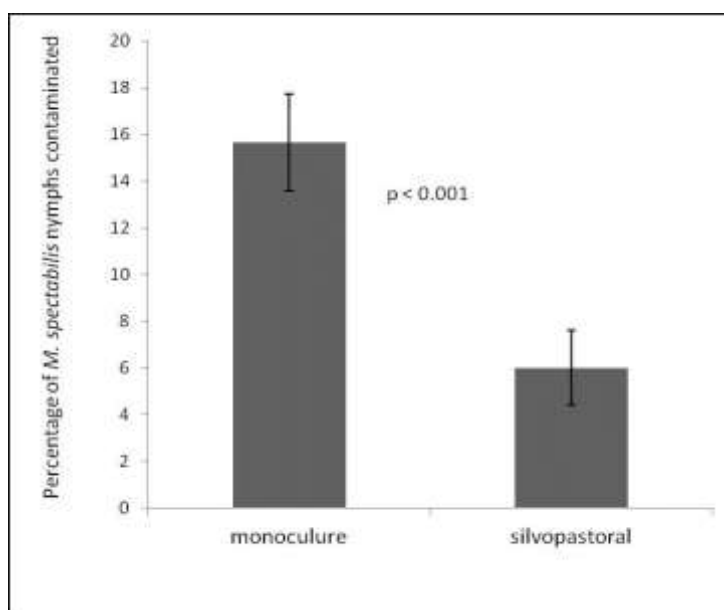


Figure 5 - Percentage of *Mahanarva spectabilis* nymphs contaminated by *Metarhizium anisopliae* in pastoral systems. Kruskal Wallis test at 5% probability.

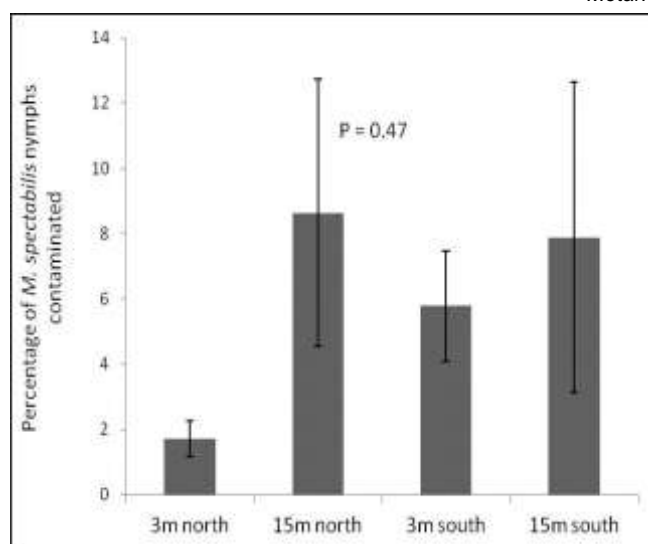


Figure 6 - Percentage of *Mahanarva spectabilis* nymphs contaminated per m² by *Metarhizium anisopliae* in silvopastoral system at distances of 3 m and 15 m on north side of central *Eucalyptus* row. Kruskal Wallis test at 5% probability

The absence of *M. spectabilis* in agrosilvopastoral system can be linked to the implementation of the palisade grass at the end of February 2014, post the soybean harvest, which is not a spittlebug host. Further, palisade grass intercropped with maize was sown in a lower density (8.22 Kg.ha⁻¹) than pasture in the monoculture (12 Kg.ha⁻¹). Thus, more solar radiation reaches the soil surface, which has a high correlation with spittlebug survival (Lohmann et al., 2010).

The shadow, necessary to protect nymphs of *M. spectabilis*, promoted by pasture intercropped with maize occurred in the end of March. At this time, *M. spectabilis* population naturally reduces due to quiescence sparked by precipitation decrease. Besides, as soybean had been cultivated early the pasture, there was no historic of spittlebug eggs in the area, showing the significant influence of crop-pasture rotation to break the pest cycle as suggested by Macedo (2009).

In more complex systems, besides food diversity and shelter, moisture and favorable temperatures are provided to establish and facilitate the development of spittlebugs (Ferreira & Marques, 1998; Wink et al., 2005) due to trees canopies which block the direct sunlight, thus reducing soil moisture loss (Koller, 1988). Although the infestation of the *M. spectabilis* nymphs was affected by solar radiation, the reduction in radiation provided by the trees canopy was not enough to significantly reduce the spittlebug infestation, as there were no differences in the distribution of the pest among the sites with and without trees shadow.

The availability of the forest component can also positively influence the nutritional value of the forage, by increasing the nitrogen concentration of the dry matter (Ribaski et al., 2003). According to Koller & Valério (1987) and Chiaradia et al. (2014), well-nourished plants provide the best pasture nutrition for spittlebugs.

Considering the evaluations started two years after both pasture systems establishment, a lower

infestation of *M. spectabilis* in pasture monoculture indicates the relation pathogen-host (fungus-spittlebug) reached a balance and that system offers biotic and abiotic conditions for both organisms.

Eucalyptus may release metabolites with inhibitory activity to plants, animal and microorganisms (Yang et al., 2017; Rieff et al., 2016; Mendes et al., 2013). Those compounds are released in soil during decomposition of leaves, barks and branches or exudates released by the roots, affecting plants, soil microfauna (Rieff et al., 2016) and microorganisms.

Zhang et al. (2010) discovered that *Eucalyptus* plants ranging from 2 - 4 years old express the peak of those inhibitory metabolites. This study reinforces our results since the trees in our experiment were three years old. Mendes et al (2013) confirmed in their review about rhizosphere microbiome, compounds released in soil by plants may affect spore germination and fungal growth. Santos et al. (1998) also reported that *Eucalyptus* species produce metabolites that inhibit the growth of soil fungi.

Further studies are required on inhibitory effects of these metabolites on *M. anisopliae* to understand the interactions of these integrated systems with the soil microbiota (Rizvi et al., 1999; Sobrero, 2004; Albach et al., 2010).

Although nymph infestation was higher and the percentage of infected nymphs was lower in silvopastoral system, none additional sprays of *M. anisopliae* was required to maintain the spittlebug population below its threshold action as well as in monoculture pastures. Crocorno (1990), Alves (1998); Pereira et al. (2008) report that the effectiveness of a biopesticide based on the microorganisms is good if accurately performed during the first infestation peaks as we observed in our study.

Conclusion

According to the results in this study, we conclude that agrosilvopastoral system with soybeans in the summer crop and maize intercropped with *U. brizanta* (var. marandu) was more sustainable, within the parameters evaluated, due to unfavorable condition for the multiplication of *M. spectabilis*. Therefore, there was no need for *M. anisopliae* sprays, subsequently decreasing the production costs. In addition, although the forest component, *Eucalyptus* negatively influenced *M. anisopliae*, its control was effective enough to maintain the spittlebug population under its action threshold.

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