Interaction between tall fescue infected with endophyte and Lotus tenuis under two defoliation frequencies


ABSTRACT

The toxicity of tall fescue infected with asexual endophyte Epichloë coenophiala can be attenuated when the species is sown in mixture with legumes. The aim of this work was to simulate the establishment of pastures of infected tall fescue (Fa), both pure and consociated with Lotus tenuis (Lt), and to evaluate the effect of two defoliation frequencies on aerial biomass production and the number of tillers in Fa and stems in Lt during the year of implantation. The experiment was conducted outdoors in plastic containers (0.6 m x 0.4 m x 0.2 m). On 24 February 2015, 52 seeds of infected Fa and 12 seeds of Lt were sown in each container for the mixed pastures (i.e. 64 plants in total), and the same number of seeds of each species was used for the respective monocultures (i.e. 52 and 12 plants in Fa and Lt monocultures, respectively). The controlled experimental factor was the defoliation frequency (70 mm cutting height): high and low (every 7-9 and 14-21 days, respectively, the interval depending on the season). A completely randomized experimental design with 3 replicates and repeated measurements in time was used (18 containers in total). Accumulated aboveground harvested biomass of each container and the number of tillers of Fa and stems of Lt plants were determined during the experimental period. Accumulated biomass of Fa and Lt in monocultures and mixtures during the experimental period varied depending on the defoliation frequency and/or type of pasture. In general, and according to the growing season of the species, at the beginning of the experimental period Fa monocultures and mix pastures accumulated more biomass than Lt monocultures, while the opposite took place by the end of it. Tillers density in Fa and stems density in Lt for both monocultures and mix pastures were not affected by defoliation frequency, except by the end of the experimental period when the stems density in Lt monocultures was higher than the observed in mix pastures. Conversely to what it was observed previously, at the end of the experimental period tillers density in Fa and stems density in Lt, for both monocultures and mix pastures, tended to be higher with low than with high defoliation frequency.

Keywords: Schedonorus arundinaceus, Epichloë coenophiala, legume, competition.
INTRODUCTION

The presence of asexual endophytes Epichloë spp. (Leuchtmann et al., 2014), former Neotyphodium spp. (Glenn et al., 1996) in tall fescue pastures, Schedonorus arundineus (Schreb.) Dumont (= Festuca arundinacea Schreb.) (Hoveland et al., 1999; Clay and Schardl, 2002) benefits grasses in their growth and grants tolerance to biotic and abiotic stresses (Malinowski and Belesky, 2000; White and Torres, 2009; Ormacini et al., 2013). However, due to the production of toxic alkaloids, especially ergovaline (Schardl and Phillips, 1997, Evans et al., 2004), cattle, horses and sheep that eat infected tall fescue suffer from a clinical disease known as tall fescue poisoning (Bacon et al., 1977; De Battista et al., 1995). This disease causes less daily weight gain, hormonal changes, reproductive alterations, gangrenous and hyperthermic syndromes (Stuedemann and Hoveland, 1988).

Intoxication due to the intake of grasses infected with endophytes has an important economic repercussion in the United States, New Zealand and Argentina, among other countries; and large economic losses have been estimated (De Battista et al., 1995, Strickland et al., 2011). However, intoxications in livestock farms are not common in our country due to different management strategies implemented by the producers (De Battista et al., 1997). On the one hand, for example, the toxicity can be attenuated if the grass is sown in a mixture with other endophyte-free grasses (Evans et al., 2012) or legumes such as Lotus tenuis (Waldst. Et Kit. Ex Willd) and Trifolium. repens L. (De Battista et al., 1997; Manzini, 1991; Tekeli and Ateş, 2005). On the other hand, Garcia Parisi et al. (2015) found that the presence of the endophyte in Lolium multiflorum L. plants would not modify the growth or nitrogen fixation of Trifolium repens L., and that the simultaneous presence of endophytes in grasses and bacteria in legumes would be positive for the community in terms of nitrogen utilization and productivity.

In pastures mixed with legumes, the initial proportions of the species tend to change over time, favouring the biomass of free grasses (León and Oesterheld 1982; Mela, 2003) or infected grasses (Sutherland and Hoglund, 1989; Eerens et al., 1998; Hoveland et al., 1999; Vazquez de Aldana et al., 2013) at the expense of dicotyledons. This change in the proportions of the mixtures can be due to multiple causes, such as host species, species in the pasture, pasture use regime, climatic factors, nutrient availability, preferences of herbivores and competitive displacement (León and Oesterheld, 1982; Dirihan et al., 2015).

Management practices that aim at maintaining certain grass-legume ratio for grazing must consider the aforementioned factors. The competitive ability and the proportion of species in the pasture can be modulated by the defoliation frequency (Manzini, 1991; Hoveland et al., 1999; Stuedemann and Seman, 2005).

Thus, on the one hand, management practices that allow maintaining a certain proportion of the legume in the mix would reduce the toxicity of the pasture via “dilution effect” (Salminen and Grewal, 2002; Salminen et al., 2003). On the other hand, the toxicity of infected tall fescue increases with the age of the plants and with less frequent and intense defoliation (Stuedemann and Seman, 2005). Therefore, defoliation management is a simple and low-cost tool that would reduce the competitive ability of the grass, ensure the growth and persistence of the legume and, thereby, reduce the toxicity of the pastures (Salminen and Grewal, 2002; Salminen et al., 2003). However, there are few published studies that investigate the effect of the defoliation on the production dynamics of mixed pastures that include infected tall fescue and legumes.

The goal of this work was to simulate the establishment of infected tall fescue pastures, both pure and consociated with L. tenuis, and to evaluate the effects of defoliation frequency on the production of aerial biomass and the density of tillers/stems of each species.

MATERIALS AND METHODS

Experimental site

An outdoor experiment was conducted at the Balcarce Integrated Unit (Faculty of Agrarian Sciences UNMdP - Agricultural Experimental Station INTA Balcarce) 37º 45’ 47.94” S, 58º 17’ 38.82” W, 130 m a. s. l. We used tall fescue (Fa) seeds from a pasture of the Mar Chiquita district (37º 32’ S; 57º 55’ W, previously identified by Petigrosso et al., 2013), harvested on December 15, 2014 (GP 69%), and scarified seeds of L. tenuis (Lt) cultivar Chajá (GP 90%) inoculated with Rhizobium loti (Strain 733).

The condition of endophytic infection of the Fa seeds was corroborated by microscopic analysis according to the protocol used by Saha et al. (1988) and Peretti (1994). 18 plastic containers (experimental units, EU) of 0.6 m x 0.4 m x 0.2 m were used. Each contained sieved and homogenized soil from the A horizon of a typical Argiudol (53 ppm of P Bray-1; 4.3% OM and 30.1 ppm N-NO3-).

On February 24, 2015, the mix of pastures and monocultures (mesocosms) was sown. We used perforated plastic plate that allowed to place - in the Fa monocultures and in the mixes of four grooves (which were 12 cm apart) (eg planting density 200 plants/m2), and in the monocultures of Lt and in the mixes 4 seeds of Lt in each of the three rows (eg planting density 200 plants/m2) (Figure 1).

Failures in the emergence of Fa seedlings were corrected within 20 days after sowing by transplanting seedlings extra cultivated in cultivation trays. The average emergence date was March 10, 2015 (Sow-emergency: 159 °Cd). The presence of the endophyte was corroborated by microscopic analysis of one tiller per plant (Latch et al., 1984) 30 days after the emergence of the Fa seedlings.

On April 15, 2015 (173 °Cd after the emergence) a “cleaning” cut was made to homogenize the height of the EU
and from that date two levels of defoliation frequency were applied at 70 mm of height from the level of the soil: high and low (every 7-9 days and every 14-21 days, respectively, depending on the season of the year). The containers were maintained free of weeds and with adequate water availability during the experimental period.

The accumulated thermal time (°Cd) was calculated as the sum of the differences between the average daily temperatures and the base temperature (4 °C, Colabelli et al., 1998). For this, we used the air temperature records from meteorological box boxes located 1.5 meters aboveground, from the Balcarce EEA Agrometeorology Station of INTA, and which are located approximately 300 m from the experimental site. No pests or pathogen attacks were observed during the experiment.

**Determination of the accumulated aerial biomass and of the number of tillers and stems**

The first experimental cut was made on April 21, 2015 for treatments with high frequency of defoliation, and on April 28, 2015 for treatments with low frequency. A total of 13 cuts were made with hand scissors for treatments with high frequency, and 7 cuts for low frequency treatments (Table 1). Prior to each cut the number of tillers was counted in 4 Fa plants (mixed pasture and Fa monoculture), as well as primary and secondary stems in 2 Lt plants (mix pasture and Lt monoculture) which were previously selected at random and marked. In the laboratory the material of each species was separated and dried in an oven at 60 °C until constant weight.

**Data analysis**

We used a completely randomized design with three repetitions and measurements repeated over time. In total, 18 EU were established: 3 types of pasture (mix of Fa and Lt, and their respective monocultures), 2 defoliation frequencies (high and low) and 3 repetitions. Due to the difference in the number of cuts between both treatments of defoliation frequency (eg 7 and 13 values for low and high defoliation frequency, respectively), the calculation of accumulated aerial biomass with high frequency of defoliation was made by adding the quantities harvested in successive cuts in order to obtain the cumulative biomass value corresponding to the same accumulation period of the treatments.
frequency of defoliation, but rather to the type of pasture. We observed that the biomass productions of the monocultures of Fa and of the mixtures were similar and higher than those of the monocultures of Lt.

Between 1034 °Cd and 1307 °Cd there was a significant interaction between the type of pasture and the frequency of defoliation. This was due to the fact that the accumulated biomass was higher in the monocultures of Fa under low defoliation frequency. The opposite occurred in the Ly monocultures, and there were no differences between defoliation frequencies in the mixtures. On the one hand, the total biomass production accumulated at the end of the experiment (1604 °Cd) was higher in the Lt monocultures regardless of the frequency of defoliation. The biomasses accumulated in the monocultures of Fa and in the mixed pastures did not present significant differences between them, although the biomass of the Fa monoculture with high frequency of defoliation tended to be lower than the rest (p= 0.0901). On the other hand, we observed that in the mix pastures the relative contribution of Lt to the total accumulated biomass was higher with low than with high defoliation frequency (35% and 24%, respectively).

We observed that none of the main effects nor their interactions were significant (p>0.05) when comparing the biomass production of Fa in monoculture and mixed with Lt.

**RESULTS AND DISCUSSION**

**Accumulated harvested aerial biomass**

Figure 2 shows the accumulation of biomass harvested in the monocultures and in the mixture for both defoliation frequencies. A significant interaction was detected between pasture type, defoliation frequency and date of the cuts (p <0.0001). Up to 593 °Cd there were no significant differences in the production of biomass accumulated due to the frequency of defoliation, but rather to the type of pasture. We observed that the biomass productions of the monocultures of Fa and of the mixtures were similar and higher than those of the monocultures of Lt.

Between 1034 °Cd and 1307 °Cd there was a significant interaction between the type of pasture and the frequency of defoliation. This was due to the fact that the accumulated biomass was higher in the monocultures of Fa under low defoliation frequency. The opposite occurred in the Ly monocultures, and there were no differences between defoliation frequencies in the mixtures. On the one hand, the total biomass production accumulated at the end of the experiment (1604 °Cd) was higher in the Lt monocultures regardless of the frequency of defoliation. The biomasses accumulated in the monocultures of Fa and in the mixed pastures did not present significant differences between them, although the biomass of the Fa monoculture with high frequency of defoliation tended to be lower than the rest (p= 0.0901). On the other hand, we observed that in the mix pastures the relative contribution of Lt to the total accumulated biomass was higher with low than with high defoliation frequency (35% and 24%, respectively).

We observed that none of the main effects nor their interactions were significant (p>0.05) when comparing the biomass production of Fa in monoculture and mixed with Lt.
The lack of response to the frequency of defoliation found in this experiment in the monoculture of Fa during winter contrasts with the results of Hart et al. (1971) who found a higher production of Fa biomass when the frequency of defoliation was low.

However, on the one hand, the defoliation frequencies studied by these authors were more contrasting than those studied in this work. The high frequency was similar (7 days), while on the low frequency was once a month. On the other hand, the higher production of Fa monoculture at low frequency of defoliation contradicts the report of Kerrisk and Thomson (1990) who found a higher production of Fa biomass when the pasture was defoliated with high frequency. However, the high frequency was similar to the low of this experiment (15 days) and the low frequency corresponded to 30-days intervals. In this work two relatively high defoliation frequencies were applied since it has been suggested that a high frequency of defoliation could reduce ergoalkaloid concentrations in infected Fa plants and, therefore, its toxicity since the plants would allocate a high proportion of carbohydrates for regrowth, leaving less photosynthates available for the synthesis of alkaloids by the endophyte (Belesky and Hill, 1997).

The accumulated biomass of Lt was not affected in the first three cuts - neither in the monoculture nor in the mix - by the frequency of defoliation or by the type of pasture (p>0.05). Between 1034 °Cd and 1307 °Cd there was an interaction between the type of pasture and the frequency of defoliation (p<0.05) given that Lt in monoculture produced more biomass under high frequency of defoliation than under low frequency, while in mixtures no differences between frequencies were recorded. In the last cut (1604 °Cd), the accumulation of biomass of Lt was higher in the monocultures than in the mixtures (p<0.0001) and there was no significant effect of the frequency of the defoliation (p= 0.1096).

The low production of aerial biomass of Lt in the first cuts was related to the low growth rates characteristic of the species at that time of the year (Sevilla et al., 1996, Vignolio and Fernández, 2011). However, in the spring-summer period, when this species shows the highest growth rates (Colabelli and Miñón, 1993; Vignolio et al., 2010), the accumulated biomass production of Lt increased until exceeding the last cut of other pastures, regardless of the frequency of defoliation. Acuña and Cuevas (1999) also did not find a significant response to the frequency of defoliation (6 and 8 weeks between defoliation) on dry matter production in a monoculture of Lt under irrigation. The lower biomass production of Lt in mixture could be due to the greater competitive ability of the grass when compared with the legume (Muslera and Ratera, 1991, Hernández et al., 2005).

Density of infected tall fescue tillers and Lotus tenuis stems

Figure 3 shows the density dynamics of tillers of Fa and of stems of Lt in monoculture and in mixture for both defoliation frequencies. In the case of Fa, none of the main effects or their interactions was significant (p>0.05). However, we...
observed that the density of Fa tillers tended to be higher under high defoliation frequency both in monoculture and in mixture (p= 0.0720). These results coincide with those published by Parsons et al. (1983) and Lemaire and Chapman (1998) who found that pastures managed with high defoliation frequency would develop a structure with a higher density of smaller tillers than those subjected to low defoliation frequencies, which would be explained by the law of -3/2 or the size/density compensation (Matthew et al., 1995).

On the one hand, the stem density of L.t was only affected by the type of pasture (p= 0.042) and the cutting date (p<0.0001). Until 1307 ºCd there were no differences in the density of Lt stems due to the type of pasture or the frequency of defoliation, although until that time it tended to be higher under high defoliation frequency (p= 0.0802). At the end of the experiment (1604 ºCd), and coinciding with what was observed in the production of aerial biomass, the density of Lt stems was higher in monoculture than in the mixture. The lack of response of Lt to the frequency of defoliation was also reported by Acuña and Cuevas (1999) for irrigated Lt monocultures subjected to lower defoliation frequencies (6 and 8 weeks) than those of this experiment. On the other hand, and contrary to what was found in the preceding cuts, at 1604 ºCd the density of stems of Lt in monoculture tended to be higher at low frequency of defoliation (p= 0.096), probably because in the high defoliation frequency the interval the cut was too short, restricting the recovery of the leaf area and limiting the production of photosynthates, which are necessary for the sprouting of the crown buds (Colabelli and Miñón, 1994; Acuña and Cuevas, 1999).

CONCLUSIONS

Based on the results we can conclude that:

The frequency of defoliation and the type of pasture differentially affected the accumulated aerial biomass of Fa and Lt (in monocultures and mixed) during the experimental period. On the one hand, the total biomass accumulated in the monocultures of Fa and mixed pastures did not present significant differences between them, although the biomass of the Fa monoculture under high defoliation frequency tended to be lower than the rest. On the other hand, the accumulated aerial biomass of Lt in monoculture was low at the beginning of the experimental period, but exceeded that of the mixed pastures and Fa monocultures at the end of that period due to the higher growth rates of the legume in the spring.

The density of Fa tillers and Lt stems, both in monocultures and in the mixture, was not affected by the frequency of defoliation, although it tended to be higher under high defoliation frequency. Only at the end of the experiment the number of stems in the L.t monoculture exceeded that observed in the mixture with Fa, which could be due to the greater competitive ability of the grass that would negatively affect the growth of Lt and, in turn, tended to be higher with low than with high defoliation frequency, both in monoculture and in mixture and contrary to that observed in previous cuts.

The results of this experiment indicate that the pastures, mixture of high fescue and Lotus tenuis, defoliated with a frequency of approximately 15 days would tend to present a greater proportion of lotus biomass than the more frequently defoliated. These results should be corroborated in future experiments in which the ergoalkaloid content of the harvested forage should be analyzed to verify if it decreases the toxicity due to a dilution effect, in addition to the biomass production dynamics of both components of the mixture.

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