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# Plant interactions and ergovaline concentration in mixture of *Lotus tenuis* and *Schedonorus arundinaceus* infected with the fungus *Epichloë coenophiala*

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**Abstract.** *Schedonorus arundinaceus* (tall fescue) population can be infected by the endophyte fungus *Epichloë coenophiala*, responsible of livestock intoxication due to fungal alkaloids (i.e., ergovaline). The effects of ergovaline in pastures can be attenuated through dilution; this is, to interseed infected tall fescue (E+) with legumes, endophyte-free grass, or with both. Plant interaction and ergovaline concentration in mixture of tall fescue (E+) and *Lotus tenuis*, a forage legume, were investigated in replacement series experiments under defoliation conditions. The interaction between species was analysed through relative yield total (RYT) of the forage biomass, aggressivity index (AI), plant cover and ergovaline concentration. The work was performed in field conditions. Six cuttings were applied during the experimental period. Plant monocultures of each species were at a constant density of 80 pl/m$^2$, while mixtures ratios were 20:60, 40:40 and 60:20 pl/m$^2$ of *L. tenuis* and tall fescue, respectively. Mortality of *L. tenuis* and tall fescue plants was not observed. Tall fescues yield and plant cover, in monoculture and in mixture, were higher than in *L. tenuis*. RYT was higher than 1 for all mixtures. According to AI, in mixture, tall fescue was the dominant species and overcompensated the lower yield of *L. tenuis*. At the early Autumn, ergovaline concentration in monoculture was 1.637±0.594 ng/g, and in the 40:40 mixture was 0.407±0.109 ng/g. Forage production was higher in mixtures than in monocultures, and *L. tenuis* contributed to reduce ergovaline concentration. According to the results, 40:40 pl/m$^2$ of tall fescue and *L. tenuis* was the best species combination. A positive effect of *L. tenuis* on growth of tall fescue mediated by nitrogen availability could have allowed the plant coexistence. Future experiments should analyse the variation of alkaloid concentration and forage production in tall fescue pastures, according to *L. tenuis* proportion, defoliation frequency and intensity under different seasons.

[Keywords: grass, legume, competition, toxicity, replacement series]

**Resumen. Interacción de plantas y concentración de ergovalina en la mezcla de Lotus tenuis y Schedonorus arundinaceus infectada con el hongo *Epichloë coenophiala*.** Las poblaciones de *Schedonorus arundinaceus* (festuca alta) pueden ser infectadas por el hongo endófito *Epichloë coenophiala*, responsable de la intoxicación del ganado por la presencia de alcaloides fúngicos (i.e., ergovalina). Los efectos de la ergovalina en las pasturas pueden ser atenuados mediante dilución; es decir, intersembrar la población de festuca alta infectada (E+) con leguminosas, con gramíneas libres de endófitos o con ambas. En experimentos de series de reemplazo bajo defoliación se estudió la concentración de ergovalina y la interacción de la mezcla de festuca alta (E+) y *Lotus tenuis*, una leguminosa forrajera. La interacción entre las especies se analizó mediante el rendimiento relativo total (RYT) de la biomasa forrajera, el índice de agresividad (AI), la cobertura vegetal y la concentración de ergovalina. El trabajo se realizó en condiciones de campo y se aplicaron seis cortes durante el período experimental. Los monocultivos de cada especie tenían una densidad constante de 80 pl/m$^{-2}$, y las proporciones de las mezclas tenían 20:60, 40:40 y 60:20 pl/m$^2$ de *L. tenuis* y festuca alta, respectivamente. No hubo mortalidad de plantas de *L. tenuis* ni de festuca alta. Los rendimientos y la cobertura vegetal de festuca alta, en monocultivo y en mezcla, fueron mayores que en *L. tenuis*. El RYT fue >1 para todas las mezclas. Según el IA, en la mezcla, festuca alta fue la especie dominante y sobrecompensó el menor rendimiento de *L. tenuis*. A principios del otoño, la concentración de ergovalina en el monocultivo fue de 1.637±0.594 ng/g, y en la mezcla 40:40 fue 0.407±0.109 ng/g. La producción de forraje fue mayor en las mezclas que en los monocultivos y *Lotus tenuis* contribuyó a reducir la concentración de ergovalina. Según nuestros resultados, la mezcla 40:40 pl/m$^2$ de festuca alta y *L. tenuis* fue la mejor combinación de especies. Esta coexistencia podría explicarse por un efecto positivo de *L. tenuis* sobre el crecimiento de festuca alta mediado por la disponibilidad de nitrógeno. Futuros experimentos deberían analizar la variación en la concentración de alcaloides y la producción de forraje en pasturas de festuca alta de acuerdo a la proporción de *L. tenuis* y la frecuencia e intensidad de defoliación en diferentes estaciones.

[Palabras clave: gramínea, leguminosa, competición, toxicidad, series de reemplazo]

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## INTRODUCTION

Tall fescue, *Schedonorus arundinaceus* (Schreb.) Dumort (synonymous *Festuca arundinacea* Schreb.), *Lolium arundinaceum* (Schreb.) S. J. Darbyshire and *Schedonorus phoenix* (Scop.) Holub is the most important cool-season perennial forage grass used in different countries as Argentina, Australia, Chile, New Zealand, United States and Uruguay (Cid et al. 2011; Petigrosso et al. 2013; Scheneiter et al. 2015; Hume et al. 2016; Garcia et al. 2017; Petigrosso et al. 2019). Tall fescue plants and seeds can be infected by the endophytic fungus *Epichloë coenophiala* (synonymous *Neotyphodium coenophialum*, Leuchtmann et al. 2014). The hyphae grow intercellularly within the stems, leaf sheaths and seeds. Endophyte reproduction is asexual and it is transmitted vertically from host mother plants to seeds (Philipson and Christey 1986; Clay and Schardl 2002; Torres and White 2010; Gundel et al. 2011; Zhang et al. 2017).

The endophyte confers many benefits to the grass infected (here after E+) as resistance to drought, salinity and protection from vertebrate and invertebrate herbivores (Malinowski and Belesky 2000; Torres and White 2010; Saikkonen et al. 2013; García Parisi et al. 2014; Hume et al. 2016). Tall fescue (E+) is the major cause of losses to livestock production in pastures and grasslands of different countries (e.g., Argentina, Australia, New Zealand and USA) (De Battista et al. 1997; Hume et al. 2016). *Epichloë coenophiala* synthesizes mycotoxins, ergovaline, an alkaloid that affects negatively the cattle, sheep and horses health that eat infected plants (Foote et al. 2011). The animal pathological symptoms are gangrenous ergotism (fescue foot), elevated body temperature, salivation, decrease in the livestock productivity and poor reproductive performance (Jacobson et al. 1969; Schmidt and Osborn 1993; De Batistta et al. 1997; Strickland et al. 2011; García et al. 2017; Petigrosso et al. 2019).

Toxicosis risk can be attenuated through dilution; this is, tall fescue (E+) pasture interseeding with other endophyte-free grass and/or forage legumes as *Trifolium pratense*, *Trifolium repens*, *Medicago sativa*, *Lotus* spp. and *Onobrychis viciifolia* (Malinowski et al. 1999; Hume et al. 2016; Villalba et al. 2016). In the Flooding Pampa (Buenos Aires, Argentina), tall fescue and *L. tenuis* are sown in pastures and the interactions between both species have been studied about forage yield, *L. tenuis* seedling survival and biological nitrogen fixation (Sevilla et al. 1996; Refi and Escuder 1998; Petigrosso et al. 2018). There is a lack of field studies and basic information about the yield and mycotoxins production of tall fescue (E+) in mixture with legume. Because in mixture plant interactions can be influenced by defoliation, competition, plant density and proportion, the replacement series experiments are appropriated to explore the yield and mycotoxins productions in the mixture of *L. tenuis* and tall fescue (E+) under different experimental conditions (Weigelt and Jolliffe 2003).

The objective of this work was to evaluate the plant interaction between *L. tenuis* and tall fescue (E+) growing in a replacement series experiment under defoliation conditions. We determined 1) plant yield, 2) plant cover, 3) indices of plant competition and 4) ergovaline concentration.

## MATERIALS AND METHODS

### *Field site*

The experiment was conducted at the Unidad Integrada Balcarce (Estación Experimental Agropecuaria, Instituto Nacional de Tecnología Agropecuaria Balcarce - Facultad de Ciencias Agrarias-UNMdP, Buenos Aires, Argentina; 37°45′ S - 58°18′ W) on a well-drained, Typic Argiudoll soil (Soil Survey Staff-USDA 1999). Tests on the upper 0.15 m of soil indicated pH (soil: $H_2O$, 1:2.5) 6.53, organic matter content 6.25%, phosphorus (by the Bray 1 method) 12.04 mg/g, and $NO_3$-N 10.51 ppm. Climate is temperate, humid-subhumid. Annual average and median precipitations from 1989 to 2015 were of 908 and 636 mm, respectively.

### *Plant species*

The seeds of tall fescue were collected in a pasture, at the "Arroyo Grande", Mar Chiquita county, Buenos Aires, Argentina (37°32′ S - 57°55′ W). The endophyte presence in the seeds was 100%, diagnosed through the microscopic examination of seedlings (Belanger 1996). *Lotus tenuis* seeds were of cv. Chajá. Seed germination test and purity analysis were determined according to ISTA (2008). Seed germination was 75% and 86% for *L. tenuis* and tall fescue, respectively. *Lotus tenuis* seeds were scarified with sand paper to break physical dormancy and inoculated with *Rhizobium loti*, $N_2$-fixing strain 733 (Vignolio et al. 2017).

*Land preparation and experimental design*

The experiment was performed using replacement series. Fifteen plots, three combinations of mixtures: 40:40; 20:60; 60: 20 pl/m$^2$ of *L. tenuis* and tall fescue plus two monocultures of 80 pl/m$^2$ of each species x 3 replicates, were performed. The plots of 2.00 m length by 1.05 m width were ploughed and hand-raked to produce a fine seedbed and separated by paths 0.60 m width. The mixture and monocultures were arranged in a randomized design. Plant densities were the same recommended in *L. tenuis* (Vignolio et al. 2017) and tall fescue (Petigrosso et al. 2013, 2018) and they were determined through hand thinning. The seeds were hand-sown on 24 March 2016 at 17.50 cm row spacing of plants. Six rows were sown, three rows by species arranged alternately. Seedling emergence was on 12 April 2016.

*Plant cover*

Seven months after seedling emergence, plant cover was determined by using a Kodak EasyShare (Model 550) colour digital camera with a spatial resolution of 3072x2048 pixels. The camera was positioned vertically 1 m above the top of the plant canopy, perpendicular to the ground. Two fixed photographs on a surface of 2500 cm$^2$ were taken in each plot from middle spring, 13 October 2016, until winter, 11 July 2017. The images were analysed by using a program CobCal v 2.1 (Ferrari et al. 2009). The percentage plant cover for each plot was obtained by averaging two values for images.

*Plant yield*

Aerial plant yield was harvested within each plot by cutting with hand scissors to 7 cm above ground level from a central area of 1 m$^2$ (1.43 m length by 0.70 m width). The samples were transported to the laboratory in labeled plastic bags, the species were separated and the dry weight (60 °C until constant weight) was determined.

During experimental period, 6 harvests were carried on, from 11 November 2016 to 11 July 2017. The harvests were according to accumulated growing degree-days (AGDD) and the rainfall; it was each 590±240 °C/day. Accumulated growing degree-days was calculated as:

$$AGDD = \sum T$$

being T the sum of the mean daily temperature. It was calculated as:

$$T = [((Max.\ T. + Min.\ T.)/2) - Tb]$$

where Max. T. and Min. T. were the maximum and minimum temperatures, respectively. Tb is the base temperature of 4 °C, which is approximately the same for both species (Colabelli et al. 1998; Vignolio et al. 2017). Negative values were not included in the calculation.

*Plant survival*

The plant survival was evaluated *in situ*, by the human eye and with the photographs used to determine the plant cover.

*Indices of plant competition*

The relative yield total (RYT) was used to estimate the complementarity in the use of resource or competition. RYT was calculated as the sum of the relative yield of each species growing in mixture (Weigelt and Jolliffe 2003):

$$RYT = RYij + RYji;$$

$$RYij = Yij / Yii, \qquad RYji = Yji / Yjj$$

where RYij is the relative yield of species i in mixture with species j. RYji is the relative yield of species j in mixture with species i. Yij is the yield of the species i growing in mixture with species j. Yii is the average yield of the species i growing in the three monoculture. Yji is the yield of the species j growing in mixture with species i. Yjj is the average yield of the species j growing in three monoculture. Yii and Yjj, were calculated as the average yield of each species growing in the three monocultures; and Yij and Yji, as average yield of each species growing in the three mixtures of the different combinations. RYT value 1 indicates that the two species make equal demands on the same limiting resources. RYT values less than 1 indicate mutual antagonism species. RYT values greater than 1 indicate advantage of mixture over monoculture because the species make different demands on the resources. Also, the yield gained by one species can be higher than the lost by the other species and in this case the RYT is greater than 1 (Weigelt and Jolliffe 2003).

Aggressivity index (AI) was used to estimate the relative yield increases in the i species over j one in mixture (Doubi et al. 2016). AI for i and j species was calculated as:

$$AIi = (Yij / Yii\ Xij) - (Yji / Yjj\ Xji)$$

$$AIj = (Yji / Yjj\ Xji) - (Yij / Yii\ Xij)$$

where Xij is the sown proportion of i in mixture with j and Xji the sown proportion of j in mixture with i. If AIi or AIj=0, both species are equally competitive; if AIi is positive and AIj negative, i is dominant species, whereas if AIi is negative and AIj positive, j is the dominant species.

### *Ergovaline concentration*

Ergovaline concentration in aerial biomass of tall fescue monoculture and mixture were determined at the Laboratorio de Contaminantes Químicos del Instituto de Tecnología de Alimentos, CIA-INTA Castelar (Buenos Aires, Argentina). The samples were prepared according to the method of Shelby et al. (1997). The ergovaline was determined in the biomass harvested at beginning of summer, on 3 January 2017 and at beginning of autumn, on 22 March 2017. Twenty grams of dry matter were used and the mixtures were prepared according to the biomass proportion of each species growing in the plot. These seasons were selected because it was reported that ergovaline concentration was different in tall fescue plants (Belesky et al. 1988; Cagaš et al. 1999).

### *Meteorological report*

Data of rainfall, maximum and minimum air temperature, solar radiation, evapotranspiration and relative humidity during the experimental period were provided by the weather station of the Unidad Integrada Balcarce (Table 1).

### *Experiment maintenance*

On 13 October 2016 all plots were cut to 7 cm above ground to homogenize the crops and the biomass was not considered in the calculus. Plots were hand-weeded without modifying the plant architecture and plant cover. No symptoms of water stress were observed in the experiment. All plots were irrigated during the months of maximum evapotranspiration (Table 1).

### *Data analysis*

Total yield, plant cover and ergovaline concentration data were analysed using analysis of variance (ANOVA). When ANOVA was significant, the means were separated by Tukey test at 5% level of probability. The RYT was analysed using three data for each mixture combination. Plant cover was analysed using a repeated-measures procedure (Scheiner and Gurevitch 2001). Data AI was calculated using average yield of the species growing

**Table 1.** Monthly means of maximum (T. Max.) and minimum (T. Min.) air temperature, relative humidity (RH), and accumulated irrigation, rainfall (PP), evapotranspiration (ETP) and radiation (Rad.) of years 2016 and 2017. Data were provided by the EEA INTA Balcarce meteorological station.

**Tabla 1.** Medias mensuales de la temperatura máxima (T. Max.) y mínima (T. Min.) del aire, humedad relativa (RH) e irrigación acumulada, precipitaciones (PP), evapotranspiraciones (ETP) y radiación (Rad.) de los años 2016 y 2017. Los datos fueron proporcionados por la estación meteorológica de la EEA INTA Balcarce.

| | T. Max. (°C) | T. Min. (°C) | RH (%) | PP (mm) | Irrigation (mm) | ETP (mm) | Rad. ($MJ.m^{-2}.day^{-1}$) |
|---|---|---|---|---|---|---|---|
| Year 2016 | | | | | | | |
| Mar. | 25.57 | 10.95 | 72.00 | 52.50 | 30 | 104.60 | 16.56 |
| Apr. | 19.66 | 9.53 | 80.83 | 81.80 | 0 | 55.90 | 9.15 |
| May. | 14.22 | 5.92 | 85.64 | 73.50 | 0 | 26.80 | 5.41 |
| Jun. | 12.93 | 4.06 | 84.73 | 32.80 | 0 | 22.70 | 5.71 |
| Jul. | 13.20 | 4.21 | 82.87 | 52.10 | 0 | 27.10 | 6.08 |
| Aug. | 17.33 | 4.18 | 72.93 | 3.50 | 0 | 56.40 | 10.77 |
| Sep. | 17.55 | 5.57 | 77.53 | 75.60 | 0 | 67.80 | 12.30 |
| Oct. | 10.91 | 8.07 | 76.12 | 51.50 | 0 | 89.60 | 14.96 |
| Nov. | 25.31 | 9.46 | 60.46 | 38.40 | 80 | 143.80 | 21.74 |
| Dec. | 31.00 | 13.85 | 53.00 | 43.80 | 100 | 201.30 | 24.05 |
| Year 2017 | | | | | | | |
| Jan. | 30.58 | 13.98 | 56.77 | 98.70 | 50 | 185.10 | 22.93 |
| Feb. | 27.36 | 16.92 | 78.35 | 82.80 | 10 | 107.50 | 16.54 |
| Mar. | 23.73 | 13.21 | 74.49 | 87.70 | 0 | 92.80 | 16.08 |
| Apr. | 20.52 | 10.78 | 78.93 | 249.00 | 0 | 56.50 | 9.62 |
| May. | 17.43 | 7.38 | 83.51 | 36.20 | 0 | 32.60 | 7.57 |
| Jun. | 14.20 | 5.23 | 81.73 | 86.70 | 0 | 27.00 | 5.88 |
| Jul. | 14.40 | 5.24 | 85.32 | 62.30 | 0 | 30.20 | 5.78 |

in monocultures and in the mixtures. All analyses were performed using the open source statistical software R (R Development Core Team 2018).

## Results

### *Plant cover*

Plant cover was significantly different among mixture ($F_{4,10}$=119, $P$=0.0001) and time ($F_{11,110}$=219, $P$=0.0001). Interaction between time and mixture was recorded ($F_{44,110}$=8.79, $P$=0.0001). Plant cover of tall fescue in monoculture and mixture was higher than *L. tenuis* monoculture (Figure 1). Maximum plant cover, approximately 90%, was at the end of experiment (Figure 1).

### *Plant yield*

During experimental period, no mortality of *L. tenuis* and tall fescue plants was observed. Total yield, the sum of six harvest, differed significantly among species combination ($F_{4,10}$=38.61, $P$=0.000005). Tall fescues yield, in monoculture and in mixture, was higher than in *L. tenuis* one (Figure 2). *Lotus tenuis* yield was lower growing in mixtures than monoculture (Figure 2).

### *Indices of plant competition*

The results of replacement series indicate that interspecific and intraspecific competition were different between species (Figure 3). RYT was higher than 1 for all mixtures, because tall fescue overcompensated the lower yield of *L. tenuis*. RYT was significantly different among mixture ($F_{2,6}$=6.10, $P$=0.0358). The maximum RYT value was when the species in mixture grew in the same plant density and proportion, 40:40 (Figure 3).

Values of the aggressivity index indicated that in the mixtures tall fescue was the dominant species (Figure 4). The responses of both species were different according to plant density. *Lotus tenuis* aggressivity was increasing with the increment of its plant proportion in the mixture (Figure 4). Contrarily, tall fescue was more aggressive with the reduction of its plant proportion (Figure 4).

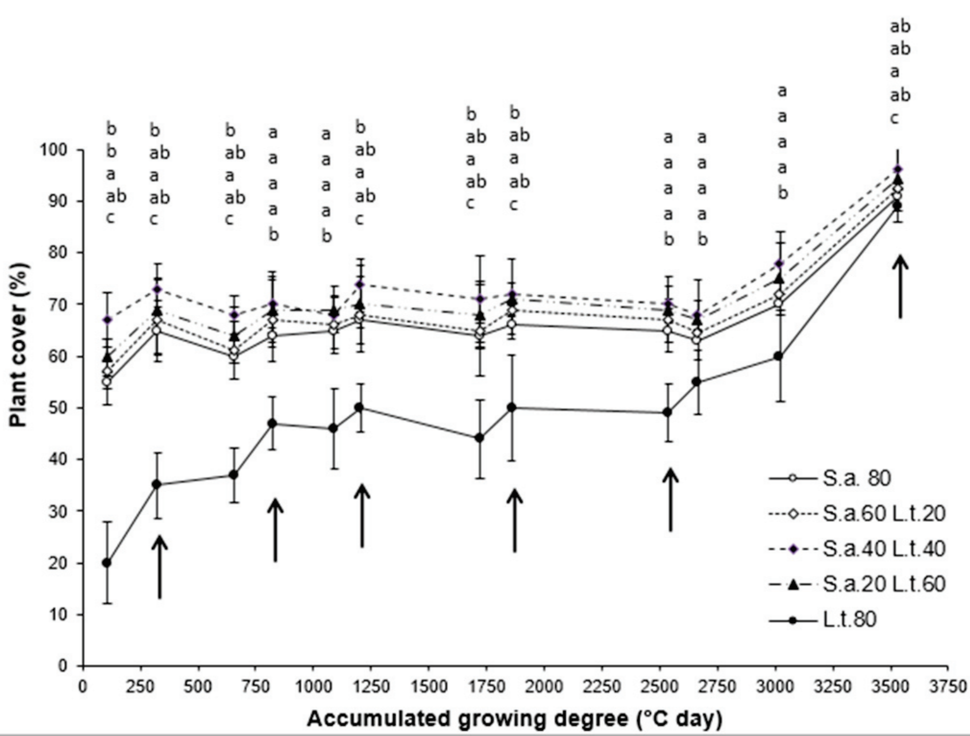


**Figure 1.** Plant cover (average±SE) of *Schedonorus arundinaceus* (S.a.) and *Lotus tenuis* (L.t.) in monoculture and in mixture at different species combinations. Total plant density was 80 pl/m². Arrows indicate the harvests. Distinct letters indicate significative differences among treatments ($P$<0.05) in a same accumulated growing degree.

**Figura 1.** Cobertura vegetal (media±EE) de *Schedonorus arundinaceus* (S.a.) y *Lotus tenuis* (L.t.) en monocultivo y en mezcla en diferentes combinaciones de especies. La densidad total fue de 80 pl/m². Las flechas indican las cosechas. Letras distintas indican diferencias significativas entre tratamientos ($P$<0.05) en un mismo grado día acumulado.

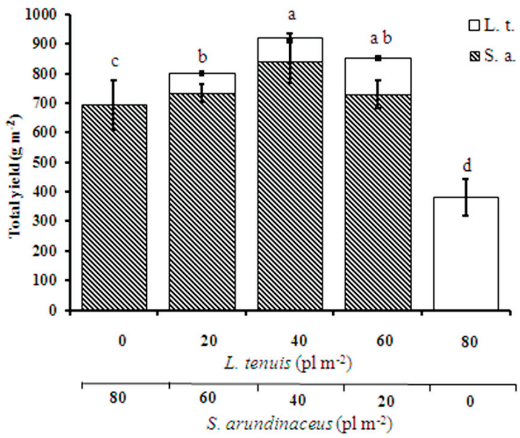


**Figure 2.** Total yield (average±SE) of *Lotus tenuis* (L.t.) and *Schedonorus arundinaceus* (S.a.) growing in monoculture and in mixture. Total plant density was 80 pl/m². Total yields with distinct letters are significantly different ($P$<0.05).

**Figura 2.** Rendimiento total (media±EE) de *Lotus tenuis* (L.t.) y *Schedonorus arundinaceus* (S.a.) creciendo en monocultivo y en mezcla. Referencias: La densidad total de plantas fue de 80 pl/m². Los rendimientos totales con letras distintas son significativamente diferentes ($P$<0.05).

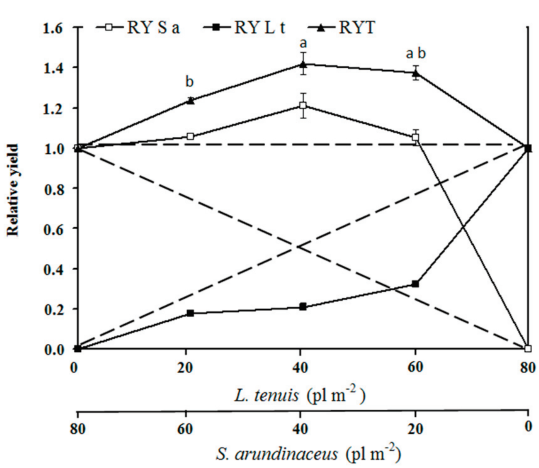


**Figure 3.** Replacement series (average±SE) showing trends in yield of *Lotus tenuis* (L.t.) and *Schedonorus arundinaceus* (S.a.) growing in monoculture and in mixture. References: RY=Relative yield; RYT=Relative yield total. Broken line is the expected RY of *L. tenuis* and *S. arundinaceus*, and dotted line is RYT when intra and interspecific interference are equal. RYT with distinct letters are significantly different ($P$<0.05). Some error bars are smaller than symbol.

**Figura 3.** Serie de reemplazo (media±EE) que muestra las tendencias en el rendimiento de *Lotus tenuis* (L.t.) y *Schedonorus arundinaceus* (S.a.) creciendo en monocultivo y en mezcla. Referencias: RY=Rendimiento relativo; RYT=Rendimiento relativo total. Línea discontinua es el RY esperado de *L. tenuis* y *S. arundinaceus*, y la línea llena es el RYT cuando la interferencia intra e interespecífica son iguales. RYT con letras distintas son significativamente diferentes ($P$<0.05). Algunas barras de error son más chicas que los símbolos.

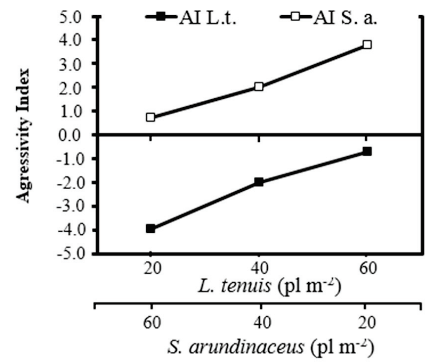


**Figure 4.** Agressivity index (AI) of *Lotus tenuis* (L.t.) and *Schedonorus arundinaceus* (S.a.) growing in mixture. Total plant density was 80 pl/m².

**Figura 4.** Índice de agresividad (AI) de *Lotus tenuis* (L.t.) y *Schedonorus arundinaceus* (S.a.) creciendo en la mezcla. La densidad total de plantas fue de 80 pl/m².

### *Ergovaline concentration*

The effect of *L tenuis* on ergovaline concentration was detected. Interaction among tall fescue (mixture and monoculture) and season was recorded ($F_{3,15}$=3.36, $P$=0.0469). Ergovaline concentration in summer was not significantly different among mixtures and monoculture, in average was 0.5166±0.0810 ng/g. In early autumn ergovaline concentration in monoculture was significantly higher, 1.637±0.594 ng/g, than in mixture 40:40, 0.407±0.109 ng/g (Figure 5).

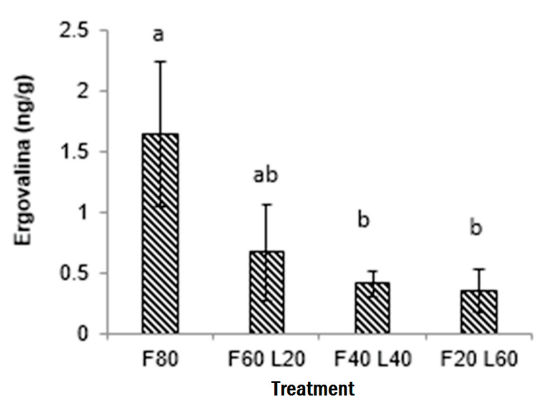


**Figure 5.** Ergovaline concentration (mean±SE) in *Schedonorus arundinaceus* growing in monoculture and in mixture with *Lotus tenuis* at the beginning autumn. Total plant density was 80 pl/m². Ergovaline concentrations with distinct letters are significantly different ($P$<0.05).

**Figura 5.** Concentración de ergovalina (media±EE) en *Schedonorus arundinaceus* creciendo en monocultivo y en mezcla con *Lotus tenuis* a principios de otoño. La densidad total de plantas fue de 80 pl/m². Las concentraciones de ergovalina con letras distintas son significativamente diferentes ($P$<0.05).

## Discussion

Tall fescue was by far more competitive than *L. tenuis*. In mixture, *L. tenuis* RY was lower than expected because it was negatively affected by tall fescue. Tall fescue RY was higher than expected, and this indicates that intraspecific competition, among tall fescue plants, was higher than interspecific one. However, RYT values were higher that one, indicating advantage in mixtures over the monocultures. Because plant mortality was not observed, it is possible some degree of niche differentiation between the species that allow the coexistence between them (Weigelt and Jolliffe 2003). The higher tall fescue yield in the species combination 40:40 pl/m² respect to monoculture, can be through reduction of the intraspecific plant competition when the grass grew with *L. tenuis*. When the plant proportion and density were the same for both species (40: 40), the RYT was maximum, 1.420. This result can be used to analyse the efficiency in the use of resources in mixture, respect to the same land areas under monoculture, considering that RYT is calculated as the land equivalent ratio, LER (Weigelt and Jolliffe 2003). In this case, the LER indicates that monoculture requires 42% more land to produce the same yield that in mixture. In other words, mixture had 42% yield advantage over the monoculture. The result provided by the RYT is consistent with aggressivity index of tall fescue, that was decreasing in mixture with the reduction of its density and proportion. We believe that an effect positive of *L. tenuis* density on the growth of tall fescue mediated by nitrogen availability could have occurred. Legumes, through symbiotic associations with N-fixing *Rhizobium* bacteria, can increase the soil nitrogen and/or it can be transferred to neighbouring plants through root exudates affecting the competitive balance among plants species and biomass production (Dulormne et al. 2003; Montesinos-Navarro et al. 2017). For example, Pirhofer-Walz et al. (2012) reported that nitrogen transferred from *Trifolium repens*, *T. pratense* and *Medicago sativa* to neighbouring plants, a mixture of grasses and dicotyledonous, was 40 kg N/ha. Quinos et al. (1998) studied the facilitative effect of *Lotus tenuis* on *Paspalum dilatatum* in a grassland of the Flooding Pampa. *Paspalum dilatatum*, in vicinity with *L. tenuis*, exhibited increase in leaf area per tiller, leaf elongation rate and slower leaf senescence, tiller production and decrease in tiller death. The results were interpreted as the result of facilitation due to nitrogen availability.

Tall fescue competitive ability can increase with the endophyte infection (Malinowski et al. 1999; Omacini et al. 2005; Hume et al. 2016), affecting the legumes yield (Malinowski et al. 1999). Vázquez de Aldana et al. (2010) reported that the root length and dry weight of *Trifolium pratense*, *T. repens*, *T. subterraneum* and *Lotus corniculatus* plants growing in a mixture with *Festuca rubra* were lower in the presence of infected plants with the endophyte *Epichloë festucae* than with the endophyte free grass. Greater competitive ability of *Festuca arundinacea* infected with *Neotyphondium coenophialum* on the growth of *T. pratense* was also reported by Malinowski et al. (1999).

The aggressivity of the species can be explained by the plant growth cycle, autumn-winter-spring of tall fescue and spring-summer-autumn of *L. tenuis* (Refi and Scuder 1998). As well as in our experiment, the pastures of tall fescue and legumes are sown in autumn, being the environmental conditions more favorable to the grass growth (Sevilla et al. 1996; Refi and Escuder 1998). This was evident when it was analyzed the species growth progress through plant cover, that was higher in tall fescue, in mixture and monoculture, than in *L. tenuis*. In spite of tall fescue aggressivity, legume mortality was not recorded. Defoliation, nitrogen sources, root distribution and morphology can reduce the competitive advantage of tall fescue and facilitate the coexistence with *L. tenuis* (Refi and Escuder 1998; Pirhofer-Walz et al. 2012; Heard and Sax 2013; Mendoza et al. 2016; Thilakarathna et al. 2016; Petigrosso et al. 2018).

The ergovaline concentration differed with the mixture and season. The highest ergovaline concentration in autumn respect to summer is consistent with the results reported by Belesky et al. (1988) in a tall fescue pasture. These authors found in tall fescue a peak of ergovaline concentration in spring and in autumn and the lower toxicity in middle summer. On the other hand, Cagaš et al. (1999) analyzed 22 ecotypes of *Festuca pratensis* and reported that ergovaline concentration was lower in autumn than summer. In summary, it is difficult to establish a seasonal pattern of ergovaline concentration in tall fescue, because alkaloid concentration differs with the genotype, season, plant growth, environmental conditions and defoliation frequency (Belesky et al. 1988; Salminen et al. 2005; Hume et al. 2016).

Acute effects, as changes in physiological functions and mortality, have been observed in animals that ate tall fescue E+ in grasslands and pastures of Flooding Pampa (De Battista et al. 1997; Garcia et al. 2017). We found that the maximum ergovaline concentration was approximately 2 ng/g in monoculture of tall fescue, being equivalent to 0.002 μg/g. The prolonged consumption of low amounts of tall fescue infected induce chronic toxicity and the effects can be reduction of weight gain and low reproductive efficiency (Evans et al. 2004; Evans et al. 2012). The threshold level of tall fescue toxicosis symptoms differs with the animal species, physiological state and age. For example, in cattle, horses and sheep, the levels of ergovaline that produce clinical disease were reported among 0.4-0.7 μg/g, 0.3-0.5 μg/g and 0.8-1.2 μg/g, respectively (Vázquez de Aldana et al. 2001). Ergovaline production in autumn mixture was lower than expected. For example, in monoculture, 80 pl/m$^2$ of tall fescue, ergovaline production was in average 1.637 ng/g. However, in mixture, 40 pl/m$^2$, tall fescue and 40 pl/m$^2$ of *L. tenuis*, ergovaline concentration was 0.407 ng/g. In other words, ergovaline production was 0.411 ng/g less than expected. In this mixture, *L. tenuis* contribution was 10% of total yield. We do not have an explanation on the subject. However, the lowest alkaloid concentration in mixture could arise by dilution, if nitrogen transferred from legume increases the growth of the grass more than the growth of the fungus (Rasmussen et al. 2007).

Our results are encouraging; because forage production was higher in the mixtures than in monocultures and *L. tenuis* can contribute to reduce ergovaline concentration and the risk of animal toxicosis. According to our results, 40:40 pl/m$^2$ of tall fescue and *L. tenuis* was the best species combination to obtain higher yield and lowest ergovaline concentration. Although in mixture tall fescue was more competitive, *L. tenuis* plant mortality was not recorded. We are interested in investigating in futures experiments how the alkaloid concentration varies in the tall fescue pastures according to *L. tenuis* proportion and defoliation frequency and intensity under different seasons.

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