Journal of Arid Environments 134 (2016) 122–124

Contents lists available at ScienceDirect

Journal of Arid Environments

journal homepage: www.elsevier.com/locate/jaridenv

Short communication

The role of grazing intensity and preference on grass-fungal endophyte symbiosis in a Patagonian steppe

Ignacio M. Hernández-Agramonte*, María Semmartin

IFEVA-Facultad de Agronomía UBA-CONICET, Av. San Martín 4453, C1417DSE Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 21 June 2016
Accepted 20 July 2016

Keywords:
Neotyphodium
Epichloë
Grazing intensity
Symbiosis
Plant palatability
Grasses
Toxicity

ABSTRACT

The symbiosis between cool-season grasses and fungi of the genus *Epichloë* is widespread, but highly variable in natural ecosystems. Biotic and abiotic controls underlying its distribution patterns have been difficult to establish, but might be related to a greater capacity of infected plants to deal with drought and herbivory. In this study we examined the relationship between grazing intensity and symbiosis frequency in plant species differentially preferred by herbivores. In all the grass species found in the studied Patagonian steppe, we determined the infection frequency along a grazing gradient by sheep (ungrazed, moderate- and intense-grazing). Three out of five preferred species and one out of four non-preferred species presented the symbiosis. Within the group of species having the symbiosis, intense grazing decreased the infection frequency of the preferred species while the frequency of the non-preferred species remained constant. These changes of infection frequency were correlated with changes in tiller biomass of hosts suggesting that symbiosis frequency could depend on overall plant performance. Our results suggest that high grazing intensity could weaken the relationship between these endophytes and their palatable hosts, eventually making them even more vulnerable to further grazing.

The asymptomatic symbiosis between cool-season grasses and the asexual endophytic fungi from the genus *Epichloë* is globally widespread in temperate ecosystems (Semmartin et al., 2015). These endophytes reside in the aboveground tissue and, in contrast to the most common horizontally transmitted fungal endophytes, they reproduce and transmit vertically through plant seeds. They are therefore viewed as strong mutualists since endophyte and host fitness are tightly linked (Clay and Schardl, 2002). Because several of these grass-fungus combinations were described to make the host more competitive, tolerant to drought, and deterrent or even toxic to herbivores, this symbiosis has been intensively investigated in forage and turf grass breeding programs, largely in *Lolium perenne* and *Festuca arundinacea* (Clay and Schardl, 2002). In contrast, our understanding of its impact on wild grass populations is considerably more limited.

The triple interaction among fungi, grasses and vertebrate herbivores in non-agricultural systems is highly complex and remains poorly understood (Huitu et al., 2014; Semmartin et al., 2015). Herbivores defoliating symbiotic plants may impair the growth of both plants and endophytes, particularly under conditions of overgrazing. In turn, endophytes synthesize metabolites whose effects on herbivores may vary from a barely detected deterrence to apparent toxicity (Clay and Schardl, 2002; Schardl et al., 2013; Luo et al., 2015). Finally, grazed plants, particularly those preferred by herbivores, due to their high digestibility, may benefit from the endophyte's defensive role. Hence, the persistence and higher endophyte frequencies observed in the field as grazing intensity increases are viewed as a sign of the defensive role of endophytes (Bazely et al., 1997; Granath et al., 2007; Rudgers et al., 2016). However, grazing intensity and endophyte frequency are not always correlated (Bazely et al., 1997; Gundel et al., 2009) denoting the complexity and context dependency of the final output of this triple interaction.

Among grass species, grazers usually prefer those with lower lignin, higher protein content and higher digestibility (McNaughton, 1984). Nevertheless, these species may be less preferred when harboring epichloid endophytes (Huitu et al., 2014). In the Occidental District of the Patagonian steppe, grasses with high and low digestibility coexist, and the abundance and individual biomass of the former group strongly decrease with grazing intensity (Perelman et al., 1997; Oñatibia and Aguiar, 2016). In spite of the relevance of the endophyte-grass association in a

* Corresponding author.
E-mail address: ihernandez@agro.uba.ar (I.M. Hernández-Agramonte).

http://dx.doi.org/10.1016/j.jaridenv.2016.07.006
0140-1963/© 2016 Published by Elsevier Ltd.

context of overgrazing, no study has addressed the effects of grazing on the frequency of this symbiosis in the Patagonian steppe, and very little is known from other ecosystems. We investigated the relationship between grazing intensity and the frequency of epichloid fungi in grass species differentially preferred by grazers. Because endophyte frequency may be affected by stress, recurrent defoliation of preferred species may impact host biomass and endophyte growth and transmission. Therefore, we predict that grazing intensity will be more negative on endophyte frequency and host biomass of preferred species than of less preferred ones.

The study was carried out at a site located in Río Mayo, Argentina (45° 24″ lat S and 70° 15″ long W), where the climate is semiarid, with an annual precipitation of 154 mm concentrated in winter, and mean temperatures in winter and summer of 2 °C and 14 °C respectively. The study area is dominated by tussock grasses and shrubs, historically grazed by guanacos (*Lama guanicoe*) and, for the last 100 years, by sheep. Intense and continuous grazing has generated extended degradation in the region, denoted by the increase of bare soil and cover of shrubs and non-preferred grasses (Perelman et al., 1997; Oñatibia and Aguiar, 2016).

We sampled all grass species in the steppe, as they were potential hosts of epichloid endophytes, in areas subjected to three different grazing intensities (Fig. S1, supplementary information): ungrazed (grazing exclosures); moderate grazing (~ 0.2 sheep ha^{-1} $year^{-1}$) and intense grazing (~ 0.4 sheep ha^{-1} $year^{-1}$). The ungrazed level consisted of three exclosures installed in 1972, 1983 and 1994, of 2.5 ha, 5 ha and 3 ha respectively, with a distance among them ranging from 1 to 10 km. Each of moderate and intense grazing levels consisted of 3 paddocks of ~200 ha, with the corresponding grazing regime maintained for at least 35 years (see details in Oñatibia and Aguiar, 2016).

In January 2012, a total of 456 plants were collected from the sites described above. From each paddock we randomly collected at least 6 plants of each grass species (except for *Bromus setifolius* which was not found in any of the intense grazing condition, see below, Table 1) that were used to estimate the endophyte infection frequency, based on the proportion of infected plants in the sample. For each plant, a minimum of 20 tillers and all available seeds were collected to detect infection. Plants were kept in nylon bags at 5 °C until processing for endophyte detection and tiller biomass. Tiller bases and seeds were stained with Rose Bengal and inspected under a microscope. Positive samples showed non-branching intercellular hyphae (Bacon and White, 1994). The infection frequency of a species in a given paddock was defined as the proportion of infected plants in the corresponding sample. Even though we did not perform fungal isolation assays, the five species found to be endophytic had been described and/or isolated previously (Iannone et al., 2011). After endophyte detection, we obtained an estimation of the individual performance of grasses under each grazing level by weighing the basal portion of tillers of the three species included in the analysis of endophyte frequency. Then, we measured the dry weight of the basal 6 cm for *Hordeum comosum* and *Poa lanuginosa* and, 10 cm for *Festuca argentina.*

During sampling we had constraints with the estimations of *Hordeum comosum* which, in the moderate grazing condition, was only determined in one of the three paddocks, and with *Festuca argentina* which, in the intense grazing condition, was only determined in one of the three paddocks. *Bromus setifolius* was not found in any of the intense grazing paddocks, therefore, based on the samples from ungrazed and moderate grazing conditions, we could confirm this species as forming the symbiosis (Table 1), but removed it from the analysis of infection frequency. Then, we obtained an unbalanced design that, for the species forming symbiosis (except *B. setifolius*), was analyzed by a glm and type III sum of squares (Shaw and Mitchell-Olds, 1993). Species and grazing were independent variables and the logarithm of the infection level was the dependant variable. In the case of plant biomass, since it was performed after endophyte detection (a destructive technique), we had a reduced amount of plants, and then pooled the samples within each grazing level. Therefore, plant biomass from the three grazing regimes was analyzed with a one way-ANOVA for each species, followed by Tukey tests for mean comparisons. The data of all the analyses had a normal distribution and homogeneity of variance. All the analyses were carried out using R statistical analysis program (R Development Core Team, 2008).

Four out of nine grass species found in this steppe formed symbiosis with epichloid fungi (Table 1). Three of them are highly preferred by herbivores (Somlo et al., 1985; Perelman et al., 1997; Adler et al., 2004) while only one, *F. argentina* despite its low lignin and high nitrogen contents (Adler et al., 2004), is not consumed presumably because of the toxicity associated with its symbiosis with *Neotyphodium tembladerae* (syn. *Epichloë tembladerae*) (Casabuono and Pomilio, 1997; Iannone et al., 2011) among other reasons such as accumulation of high amounts of lignified standing dead biomass. The species not forming symbiosis were the two preferred (*Bromus pictus* and *Poa ligularis*) and the three low digestible and non-preferred species (*Pappostipa* spp.) (Table 1).

The endophyte frequency under the three grazing conditions showed a different pattern for *Hordeum comosum, Poa lanuginosa* and *Festuca argentina*. The two preferred species showed a lower frequency under intense grazing while infection of *F. argentina* was insensitive to grazing intensity (Fig. 1). Tiller biomass of *P. lanuginosa* was significantly lower under intense grazing whereas *F. argentina* biomass was lower under exclosures. In contrast, biomass of *H. comosum* was significantly higher under excloures than under moderate or intense grazing (Table 2).

This report shows, for the first time, a negative association between grazing intensity and endophyte frequency in preferred grass species. Previous studies in Scottish islands (Bazely et al.,

Table 1
Grass species where *Epichloë* endophytes were present (bold) or absent (plain). Species were classified by preference based on Somlo et al. (1985), Perelman et al. (1997), and Adler et al. (2004). The number of plants collected per species and grazing level is indicated in the right columns.

Grass species	Preference	Number of collected plants		
		Ungrazed	Moderate	Intense
Bromus pictus	Preferred	15	8	9
Bromus setifolius	**Preferred**	16	6	0
Hordeum comosum	**Preferred**	17	8	15
Poa lanuginosa	**Preferred**	17	12	16
Poa ligularis	Preferred	21	28	21
Festuca argentina	**Non-preferred (toxic)**	13	13	10
Pappostipa humilis	Non-preferred	21	27	20
P. speciosa sub. mayor	Non-preferred	21	30	21
P. speciosa sub. speciosa	Non-preferred	21	28	22

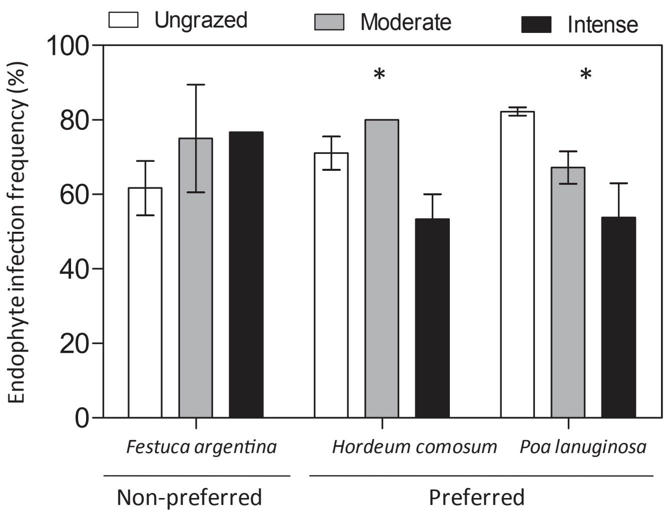

Fig. 1. Infection frequency of *Epichloë* endophytes in preferred and a non-preferred grass species across a grazing intensity gradient in a Patagonian semi-arid steppe. Vertical bars indicate mean ± 1SE and asterisks denote significant differences among grazing levels for each species, $p \leq 0.05$.

Table 2
Biomass in grams of dry matter (mean ± SE) of the basal portion of tillers of three grass species forming symbiosis with *Epichloë* endophytes along a grazing gradient. Different letters denote significant differences after Tukey tests among grazing levels within each species ($p \leq 0.05$).

Grass species	Weight per tiller (g)		
	Ungrazed	Moderate	Intense
Hordeum comosum	0.08 ± 0.01a	0.05 ± 0.01b	0.06 ± 0.03b
Poa lanuginosa	0.12 ± 0.03a 0.a03 ab	0.1 ± 0.02ab	0.09 ± 0.02b
Festuca argentina	0.17 ± 0.02a	0.2 ± 0.04b	0.23 ± 0.03b

1997) and in Sweden (Granath et al., 2007) found greater symbiosis frequency at sites grazed by wild herbivores compared to ungrazed or lightly grazed sites. In our case, grazing by domestic herbivores affected individual performance of the preferred species, a pattern largely documented in Patagonian steppes (Ares et al., 1990; Perelman et al., 1997; Oñatibia and Aguiar, 2016). Stressing environmental conditions, including overgrazing, are recognized to impair endophyte transmission (Gundel et al., 2009) and might have accounted for the reductions observed in the present study. Host biomass reduction under grazing might have been strong enough to impair endophyte frequency of the apparently non-toxic symbiosis. On the contrary, the symbiosis frequency of *F. argentina* was not affected by the grazing and its plant biomass increased under moderate and intense grazing compared to the ungrazed condition, presumably as a consequence of the light defoliation due to the toxicity and the accumulation of standing dead biomass (Casabuono and Pomilio, 1997; Iannone et al., 2011).

In conclusion, this study showed that the endophyte frequency and host performance of grass-fungi symbioses were lower in intense grazing in two apparently non-toxic symbioses, whereas the endophyte frequency remained constant and host biomass increased in a potentially toxic symbiosis. Previous studies repeatedly showed how high grazing intensity reduced the abundance of preferred species in this Patagonian steppe. Our results suggest that high grazing intensity might also weaken the association of these species with their epichloid symbionts. As a consequence, they might be even more vulnerable to further grazing. Conversely, when the association potentially confers toxicity, as in *F. argentina* in Patagonia or in *Poa huecu*, *Festuca hieronymi*, and *Festuca dissitiflora* in other steppes of South America (Iannone et al., 2011), the grass host might acquire higher resistance levels that allow them to resist higher grazing intensities.

Acknowledgements

We thank M. Omacini, P. Gundel and M. Oesterheld for their contribution in discussions and manuscript preparation. We also thank G. Oñatibia, M. Aguiar and L. Iannone for sharing their knowledge of the system, and L. Boyero, F. Reyes, P. Graff, V. Piazza and J. Rivero for their help during plant collection and analysis. We thank three anonymous reviewers whose contribution was critical to refine our ideas and to improve the manuscript. This study was funded by Agencia Nacional de Promoción Científica y Tecnológica, Universidad de Buenos Aires and Conicet and the Instituto Nacional de Tecnología Agropecuaria provided facilities in the field.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jaridenv.2016.07.006.

References

Adler, P.B., Milchunas, D.G., Lauenroth, W.K., Sala, O.E., Burke, I.C., 2004. Functional traits of graminoids in semi-arid steppes: a test of grazing histories. J. Appl. Ecol. 41, 653–663.
Ares, J., Beeskow, A.M., Bertiller, M., Rostagno, M., Irisarri, M., Anchorena, J., Defosse, G., Merino, C., 1990. Structural and dynamic characteristics of overgrazed lands of Northern Patagonia, Argentina. In: Breymeyer, A.I. (Ed.), Managed Grasslands. Ecosystems of the World, 17A. Elsevier, Amsterdam, pp. 149–175.
Bacon, C.W., White Jr., J.F., 1994. Stains, media, and procedures for analyzing endophytes. In: Bacon, C.W., White Jr., J.F. (Eds.), Biotechnology of Endophytic Fungi of Grasses. CRC Press, Boca Raton, pp. 47–56.
Bazely, D.R., Vicari, M., Emmerich, S., Filip, L., Lin, D., Inman, A., 1997. Interactions between herbivores and endophyte-infected *Festuca rubra* from the Scottish islands of St. Kilda, benbecula and rum. J. Appl. Ecol. 34, 847–860.
Casabuono, A., Pomilio, A.B., 1997. Alkaloids from endophyte-infected *Festuca Argentina*. J. Ethnopharmacol. 57, 1–9.
Clay, K., Schardl, C., 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am. Nat. 160, 99–127.
Granath, G., Vicari, M., Bazely, D.R., Ball, J.P., Puentes, A., Rakocevic, T., 2007. Variation in the abundance of fungal endophytes in fescue grasses along altitudinal and grazing gradients. Ecography 30, 422–430.
Gundel, P.E., Garibaldi, L.A., Tognetti, P.M., Aragón, R., Ghersa, C.M., Omacini, M., 2009. Imperfect vertical transmission of the endophyte *Neotyphodium* in exotic grasses in grasslands of the flooding pampa. Microb. Ecol. 57, 740–748.
Huitu, O., Forbes, K.M., Helander, M., Julkunen-Tiitto, R., Lambin, X., Saikkonen, K., Stuart, P., Sulkama, S., Hartley, S., 2014. Silicon, endophytes and secondary metabolites as grass defenses against mammalian herbivores. Front. Plant Sci. 5, 478.
Iannone, L.J., White Jr., J.F., Giussani, L.M., Cabral, C., Novas, M.V., 2011. Diversity and distribution of *Neotyphodium*-infected grasses in Argentina. Mycol. Prog. 10, 10–19.
Luo, H., Xie, L., Zeng, J., Xie, J., 2015. Biosynthesis and regulation of bioprotective alkaloids in the gramineae endophytic fungi with implications for herbivores deterrents. Curr. Mycrobiol. 71, 719–724.
McNaughton, S.J., 1984. Grazing lawns: animals in herds, plant form, and co evolution. Am. Nat. 124, 863–886.
Oñatibia, G.R., Aguiar, M.R., 2016. Continuous moderate grazing management promotes biomass production in patagonian arid rangelands. J. Arid Environ. 125, 73–79.
Perelman, S.B., León, R.J.C., Bussacca, J.P., 1997. Floristic changes related to grazing intensity in a patagonian shrub steppe. Ecography 20, 400–406.
R Development Core Team, 2008. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org. ISBN 3-900051-07-0, URL.
Rudgers, J.A., Fletcher, A.R., Olivas, E., Young, C.A., Charlton, N.D., Pearson, D.E., Maron, J.L., 2016. Long-term ungulate exclusion reduces fungal symbiont prevalence in native grasslands. Oecologia 181, 1–11.
Schardl, L.C., Florea, S., Pan, J., Nagabhuyru, P., Bec, S., Calie, J.C., 2013. The epichloae: alkaloid diversity and roles in symbiosis with grasses. Plant Biol. 16, 480–488.
Semmartin, M., Omacini, M., Gundel, P.E., Hernández-Agramonte, I.M., 2015. Broad-scale variation of fungal-endophyte incidence in temperate grasses. J. Ecol. 183, 184–190.
Shaw, R.G., Mitchell-Olds, T., 1993. Anova for unbalanced data: an overview. Ecology 74, 1638–1645.
Somlo, R., Durañona, C., Ortíz, R., 1985. Valor nutritivo de especies forrajeras patagónicas. Rev. Argent. Prod. 5, 589–605.