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Symbiosis with systemic fungal endophytes promotes host escape from vector-borne disease

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Abstract Plants interact with a myriad of microorganisms that modulate their interactions within the community. A well-described example is the symbiosis between grasses and *Epichloë* fungal endophytes that protects host plants from herbivores. It is suggested that these symbionts could play a protective role for plants against pathogens through the regulation of their growth and development and/or the induction of host defences. However, other endophyte-mediated ecological mechanisms involved in disease avoidance have been scarcely explored. Here we studied the endophyte impact on plant disease caused by the biotrophic fungus, *Claviceps purpurea*, under field conditions through (1) changes in the survival of the pathogen´s resistance structure (sclerotia) during overwintering on the soil surface, and (2) effects on insects responsible for the transportation of pathogen spores. This latter mechanism is tested through a visitor exclusion treatment and the measurement of plant volatile cues. We found no significant effects of the endophyte on the survival of sclerotia and thus on disease inocula. However, both pathogen incidence and severity were twofold lower in endophyte-symbiotic plants than in non-symbiotic ones, though when insect visits were prevented this difference disappeared. Endophyte-symbiotic and non-symbiotic plots presented different emission patterns of volatiles suggesting that they can play a role in this protection. We show a novel indirect ecological mechanism by which endophytes can defend host grasses against diseases through negatively interacting with intermediary vectors of the epidemic process.

Keywords Mutualism · Pathogen transmission · Disease avoidance · VOCs · *Epichloë occultans*

Introduction

Over recent years, there has been a growing interest in understanding how, and to what extent, the effects of plant associations with above and belowground microorganisms can propagate beyond the host, and impact other biotic interactions within the neighborhood (Borer et al. 2007; Hudson et al. 2006; Mitchell et al. 2006). For many plant species, these associations can be either transient or persistent and range from beneficial to harmful depending on the balance of costs and benefits (Afkhami et al. 2014; Newton et al. 2010; Saikkonen et al. 1998). Furthermore, the interacting organisms have complex life cycles and are simultaneously or consecutively exposed to multiple direct and indirect effects from the biotic and abiotic context. Thus, it is the network of interactions across their life stages that ultimately determine the establishment and the outcome of the plant–microbe interactions (Saikkonen et al. 1998; Thrall et al. 2007).

Cool-season grasses establish different type of intimate interactions with clavicipitaceous fungi with contrasting outcomes in terms of their net effects on hosts: *mutualistic*

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as the case of strictly seed-transmitted species (e.g., asexual *Epichloë* spp) and *pathogenic* as the case of those fungal species infecting flowers and avoiding seed formation (e.g., *Claviceps* spp). Both types of fungi are capable of producing several secondary metabolites that play defensive roles against herbivores (Schardl et al. 2013; Wäli et al. 2013) and other pathogens (Tian et al. 2008). However, little is known about their reciprocal effects when they are competing for the same host (and even the same organ) and the ways in which environmental conditions modulate the tripartite mutualist–pathogen–host interaction (Pérez et al. 2013). Moreover, no ecological studies have been conducted in order to evaluate the effects across different life-history stages of each partner.

Asexual *Epichloë e*ndophytes grow systemically and asymptomatically within host aboveground tissues, but can affect many above and belowground ecosystem components and processes (Clay and Schardl 2002; Omacini et al. 2012). Unlike other fungi that reproduce sexually, these symbionts are vertically transmitted by colonizing plant ovaries and seeds (Schardl and Leuchtmann 2004). This type of long-lasting grass–endophyte relationship modifies the chemistry and morphology of host tissues and the host interaction with the abiotic and biotic environment (Malinowski and Belesky 2000). A high percentage of studies report an improved defence of symbiotic plants at the moment of dealing with natural enemies, mainly herbivores (Clay 1993, 1996; Saikkonen et al. 2013). Endophyte presence usually reduces the abundance of different type of herbivores affecting the energy flow through food chains (García Parisi et al. 2014; Kunkel et al. 2004; Omacini et al. 2001). Furthermore, the litter produced by plants associated with endophytes can also interfere with the performance of the next generation of plants and their relationship with herbivores (Omacini et al. 2009) or other mutualistic fungi (Antunes et al. 2008).

Endophyte symbiosis can influence the rhizospheric environment of host grasses by affecting the composition and functioning of soil biota (Buyer et al. 2011; Casas et al. 2011; Matthews and Clay 2001; Rojas et al. 2016; Rudgers and Clay 2008; Rudgers and Orr 2009). Many of the endophyte-mediated effects on the soil community were recently summarized by Omacini et al. (2012). Given the small number of studies, the impact on the interaction with soil pathogens could not be included in the meta-analyses despite their ecological importance. The symbiosis with fungal endophytes may be critical for host plants to cope with diseases (Clarke et al. 2006; Pańka et al. 2013; Pérez et al. 2013; Wäli et al. 2006). An overall protection mediated by the endophyte has been observed in several pathosystems occurring at different stages of the plant life cycle (Pérez et al. 2013; Pérez et al. 2015; Tian et al. 2008; Vignale et al. 2013; Wäli et al. 2006; Welty et al. 1991). The underlying mechanisms of these effects have usually been associated with a direct impact of the endophyte on pathogen growth (e.g., inhibition or competition) and with endophyte-promoted changes on the host immune system that hinder disease progress (Hamilton et al. 2012; Wäli et al. 2006; West and Gwinn 1993; Yue et al. 2000). However, as most studies have focused on just one single plant stage and/or have been carried out under controlled and simplified experimental conditions, our understanding of the *Epichloë* endophyte effect on plant–pathogen interactions is limited (Chamberlain et al. 2014).

All endophyte-induced changes in the host grass and its environment can influence other relationships that may take place at later stages, including those with pathogens. The triple interaction among plants, beneficial endophytes and pathogens has mostly been addressed through laboratory and small-scale experiments in order to study direct effects among the interacting organisms. However, endophyte effects may differ depending on the moment, and more importantly, on the environmental conditions in which the interaction with pathogens occurs. In nature, many ecological mechanisms involving other functional groups may be taking place (i.e., fungivores, floral visitors and pathogen vectors). Many endophyte-promoted strategies, such as the alteration of plant reproduction (Gorischek et al. 2013), the interaction with insects such as floral visitors that also act as pathogen vectors (Li et al. 2014; Rúa et al. 2013; Schiestl et al. 2006) along with those changes generated in the soil, may impact on the life cycle of biotrophic fungi such as *Claviceps purpurea*. The cumulative effect of the mechanisms mentioned above along the life cycle of host plants may explain the differences observed in pathogen infection.

In this paper, we explore two ecological pathways by which a resident symbiont (here the endophyte *Epichloë occultans*) may affect the infection dynamics of another clavicipitaceous fungi: the flower-infecting pathogen *Claviceps purpurea*. Our approach takes into account a year-round life cycle of the pathogen in two contrasting scenarios defined in terms of the endophyte presence within the annual grass *Lolium multiflorum* and its legacy through host litter (Fig. 1). We hypothesize that living and dead host tissues affect the survival of pathogen sclerotia in the surrounding soil (Pre-infection mechanisms, Fig. 1a) and the pathogen infection of plant flowers by the emission of volatile compounds that repel insect vectors of *C. purpurea* spores (Infection-related mechanisms, Fig. 1b).

Methods

Plant material

Lolium multiflorum Lam. (Italian ryegrass) is an annual grass of high constancy in grasslands of the Pampa region

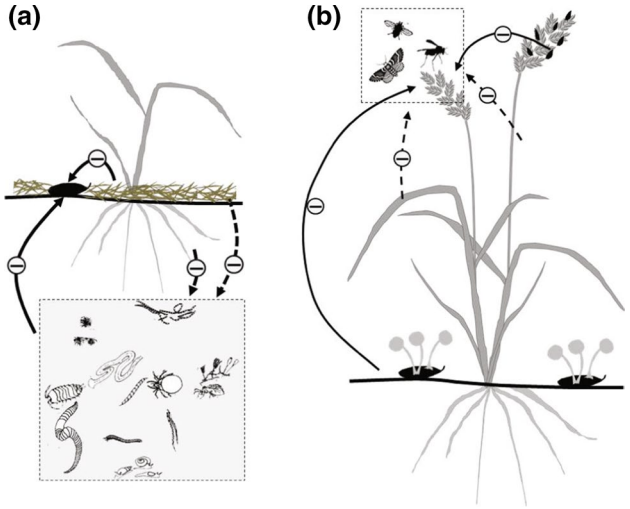

Fig. 1 Diagrammatic representation of **a** pre-infection mechanisms and **b** infection-related mechanisms by which endophytes can defend host grasses against diseases. Pre-infection mechanisms include the endophyte effects on soil biota (*dashed arrows* from the plant to soil fauna and soil microorganisms) that exert a negative effect on the survival of the pathogen's sclerotia (*arrows* connecting soil fauna and soil microorganisms with sclerotia). Infection-related mechanisms include endophyte effects on vectors (*dashed arrows* connecting plants to vectors) transporting spores from germinated sclerotia and infected flowers to healthy ones (*solid arrows* connecting germinated sclerotia and infected flowers to healthy spikes through vectors)

(Soriano 1992). Characterized as a competitive-ruderal species, its invasive behavior in pampean grasslands has been associated with the presence of the endophytic fungus *Epichloë occultans* (formerly *Neotyphodium occultans*, Omacini et al. 2009; Tognetti and Chaneton 2012). For this study, mature seeds of *L. multiflorum* were collected from a population occurring in an old-field grassland in the Pampa region, Argentina (35°55′14.70″S, 61°9′29.24″W). A preliminary evaluation established that 95% of the population was composed of endophyte-symbiotic individuals (based on microscopic observation of 100 seeds stained with Rose Bengal dye) (Bacon and White 1994). Non-symbiotic individuals from the same population were generated by treating half of the harvested seeds with the systemic fungicide triadimenol (150 g ai kg^{-1}, dose: 5 mg per gram of seed). Both treated and untreated seeds were sown in monocultures in adjacent plots of 1 m^2 in the experimental field of the Faculty of Agronomy, University of Buenos Aires. During flowering, free pollination was allowed in order to keep populations with the same genetic background. This culture process was repeated for one more generation to lessen any phytotoxic effect of the fungicide (Omacini et al. 2006, 2009). Pollen flow was allowed among the two populations with the aim of avoiding any kind of genetic divergence between them (Gundel et al. 2012). Seeds produced by these plants (F1) were used for both experiments. A hundred seeds from each subpopulation were checked to confirm the effectiveness of the fungicide treatment. Endophyte frequencies were 90 and 10% for untreated seeds (E+) and treated seeds (E−), respectively. The remaining dead material on the plots was left in situ until used for symbiotic and non-symbiotic litter treatments (from now on L+ and L− litter, respectively).

Pathogen

Claviceps purpurea (*Fr.:Fr.*) *Tul.* infects many Poaceae species and shares a common environment with its host until the moment of the infection process (Alderman 1993; Rius et al. 2014). Prior to the infection, pathogen sclerotia must survive adverse conditions in the soil in order to germinate and produce ascospores. When the appropriate conditions occur, sclerotia germinate bearing apothecial stalks containing the ascospores which will ultimately infect the host flowers (Alderman 1993; Johnston et al. 1996) (Fig. 1). From the moment of their formation and until germination, sclerotia must overcome biotic filters, such as predation by large and small animals, soil fauna and microorganisms (Feldman et al. 2008; Wäli et al. 2013) as well as abiotic filters, such as soil temperature and moisture (Dabkevičius and Mikaliunaite 2006; Johnston et al. 1996; McLaren and Flett 1998). Host infection occurs when an ascospore germinates in a plant's floret and grows profusely into the ovary. Additionally the pathogen produces "honey dew", a droplet of sweet plant sap containing fungal conidia, which can be dispersed by many agents such as wind, water or insects visiting flowers causing new infections. The result of the infection process is the replacement of the seed by fungal biomass that eventually forms a sclerotium.

The summer prior to the establishment of the experiment, sclerotia of *C. purpurea* were randomly collected from infected plants occurring at the same site where *Lolium multiflorum* seeds were harvested. Sclerotia were taken carefully from the spikes, cleaned and preserved at 20 ± 5 °C until the experiment was established. In order to test their viability, 50 sclerotia were surface sterilized by immersion in a 0.5% sodium hypochlorite solution for 2 min followed by soaking in sterile distilled water for 30 s. Later they were subjected to stratification at 5 °C for 70 days and then placed in a 23 °C chamber for 50 days (Johnston et al. 1996). The totality of the sclerotia germinated during the incubation period.

Plots establishment

Sixteen 0.7 m × 0.7 m plots arranged in eight blocks of two plots each were established in the same old-field grassland where seeds were collected. In late spring of 2011 all plant material was removed from the plots and soil was solarized

by covering the plot with black polyethylene. Thus, the contribution of neighboring populations to the seed bank and growth of other plants were avoided, and the survival of the seeds present in the seed bank was reduced. In late summer of 2012 each plot was uncovered and watered in order to promote germination and thus check the effectiveness of the solarization treatment. After 15 days, each plot was randomly sown with 5 g of symbiotic (E+) or non-symbiotic (E−) *L. multiflorum* seeds (~10 g/m^2). The seeds were then covered with a thin layer of sand and protected with nets in order to avoid their predation by birds. The nets were removed after 1 month.

Pre-infection mechanisms

At sowing time, six 5 × 5 cm tulle bags containing 10 sclerotia of *C. purpurea* were placed in each plot. One-third of the sclerotia bags were covered with 10 × 10 cm mesh bags (2 mm mesh size) containing 4 g of litter produced by E+ plants (L+); another third, with bags containing 4 g of litter produced by E− plants (L−); and the remaining third with empty bags (L0). Leaf:stem ratio of litter was 0.65 ± 0.23 and 0.47 ± 0.07 for L+ and L−, respectively ($F_{1,2} = 0.52$; $p = 0.51$). Bags containing *C. purpurea* sclerotia and the litter were harvested 9 months after plant sowing. The collection date was established in order to avoid the sclerotia germination and its subsequent decomposition. Sclerotia were removed from the bag, cleaned with a brush to remove debris and soil, and surface sterilized by immersion in a 0.5% sodium hypochlorite solution for 2 min followed by soaking in sterile distilled water for 30 s. They were later subjected to stratification at 5 °C for 70 days and placed in a 23 °C chamber for 50 days (Johnston et al. 1996). After this period the germination of sclerotia and the number of stalks were recorded.

At the end of October soil mesofauna was sampled in each symbiotic and non-symbiotic plot. A 6 cm diameter and 10 cm depth soil core was taken and kept refrigerated at 4 °C until use, 24 h later. The extraction of soil organisms was performed with modified Tullgren funnels for a period of 15 days (Crossley and Blair 1991). Adult specimens were counted and identified to order level. Abundance was calculated for each subplot as the number of individuals per gram of dry soil.

Infection-related mechanisms

At the end of October, when most of the grasses had reached their reproductive stage, but prior to anthesis, each plot was randomly divided in halves. The spikes from one of the subplots were closed with a tulle bag that and allowed the flow of pollen and spores propagated by wind and kept insects out, while the spikes from the remaining subplot were grouped emulating the effect of bagging without obstructing the entrance of vectors (V− and V+, respectively, from now on) (Neal and Anderson 2004). After the plant cycle had completed and seeds were observed (first days of December), bags were removed and all spikes per treatment were harvested. Pathogen incidence and severity were calculated for each subplot as the percentage of spikes showing signs of infection and the percentage of seeds replaced by sclerotia in each spike, respectively. Non-infected spikes were not included in the estimation of severity.

At the same moment of installing the tulle bags, the emissions of volatiles organic compounds (VOCs) were characterized in each plot with an electronic nose (AgriNose, CNEA, Buenos Aires, AR) (Branca et al. 2003; D'Alessandro and Turlings 2006). The electronic nose consists of an array of 8 × 8 tin-dioxide, non-specific gas sensors and a central processor. The chamber is made of a "neutral" and stable material, whose electrical conductance increases in the presence of reducing gases. The gases are transferred into the chamber by a pump at a fixed flow rate. Reference air and odorous samples are taken intermittently. The response of the sensor is the difference between the signal, after equilibrium in the odorous ambience, and the base line generated in pure air taken at a height of 2 meters (see methodology described by Szpeiner et al. 2009). For each measurement, the sensor was placed in the middle of the plot at half the height of the grass plants.

Statistical analyses

The number of sclerotia recovered, its germination and the number of apothecial stalks per sclerotium produced by each one in each type of plot were analyzed separately by an analysis of variance (ANOVA) in a block and split-plot design with endophyte symbiosis in living plants as the main plot and litter as the subplot. The abundance of soil organisms within each group was transformed using ln(x + 1) and analyzed using a multivariate analysis of variance (MANOVA) in a randomized complete block design.

The values of pathogen incidence and severity were compared among treatments using separate analyses of variance (ANOVA) in a block and split-plot design. Blocks were used for taking into account spatial heterogeneity; endophyte presence was the main plot level factor and vector exclusion was the subplot factor. At the end of the experiment, two tulle bags from two different blocks were found missing, thus for the analyses of incidence and severity only six blocks were taken into consideration. Volatile organic compounds emission was analyzed for each sensor separately using analysis of variance (ANOVA) in a block design.

Results

Pre-infection mechanisms

Within all treatments more than 60% of *C. purpurea* sclerotia were recovered after 240 days. The presence of endophytes in living plants or their legacy through the host litter generated no significant differences in terms of number of recovered sclerotia ($F_{\text{endophyte } 1,7} = 1.43$; $p = 0.27$: $F_{\text{litter } 2,7} = 1.36$; $p = 0.28$; $F_{\text{endophyte} \times \text{litter } 2,14} = 0.06$; $p = 0.93$; Fig. 2a) and number of apothecial stalks per sclerotia ($F_{\text{endophyte } 1,7} = 0.12$; $p = 0.74$: $F_{\text{endophyte} \times \text{litter } 2,14} = 0.03$; $p = 0.96$; Fig. 2b). A significant increase in the number of stalks was observed in sclerotia bags not covered with litter ($F_{2,14} = 3.62$; $p = 0.05$). The presence of endophytes in living plants and the signals left in their litter did not modify the abundance of the different groups within the arthropod community (Pillai trace = 0.36, $p = 0.86$; Table 1).

Infection-related mechanisms

Incidence of *C. purpurea* in non-symbiotic plots without the vector exclusion treatment doubled was double the values of the other treatments ($F_{\text{endophyte} \times \text{vector exclusion}1,5} = 35.10$; $p = 0.002$) (Fig. 3a). Differences in severity were only observed among vector-excluded and not excluded spikes in non-symbiotic plants ($F_{\text{endophyte} \times \text{vector exclusion}1,5} = 8.82$; $p = 0.031$) (Fig. 3b). In symbiotic plots, the effect of excluding vectors on incidence and severity was almost four times smaller than in non-symbiotic ones. There were no differences in spike number across treatments ($F_{\text{endophyte } 1,16} = 0.33$: $p = 0.575$).

Plant association with endophytes generated changes in the emission of volatile organic compounds that were only detected by three sensors of the electronic nose (S1:$F_{\text{endophyte}1,16} = 9.15$, $p = 0.019$; S7:$F_{1,16} = 12.92$, $p = 0.008$; S8: $F_{1,16} = 6.04$, $p = 0.043$) (Fig. 4). Additionally, a tendency was observed in three of the five remaining sensors (S2: F: 5.20, p:0.056; S3: F:5.24, p:0.055; S4: F:5.41, p:0.052) (Fig. 4).

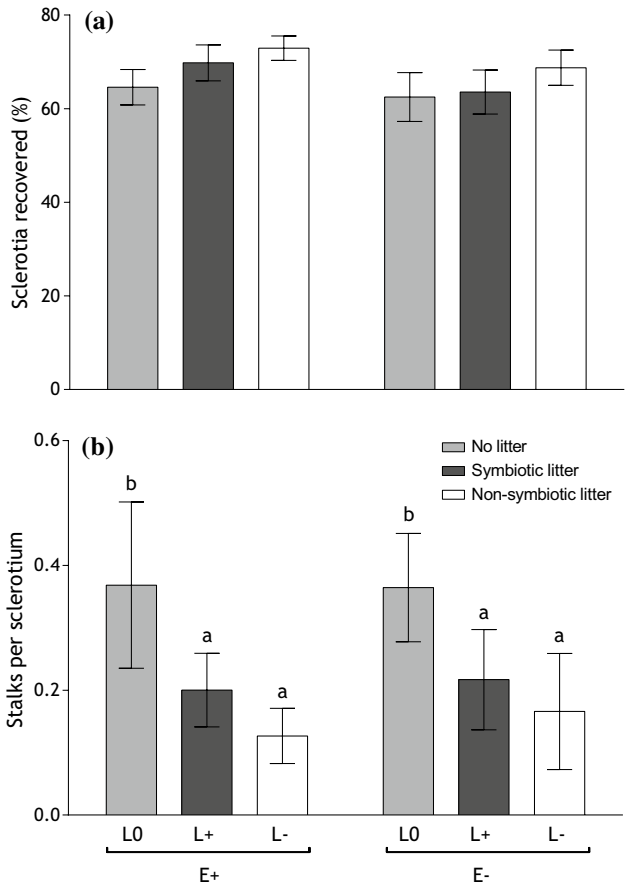

Fig. 2 **a** Recovery and **b** number of stalks per sclerotium of *C. purpurea* placed under litter bags containing senescent material from *L. multiflorum,* symbiotic (L+ *black bars*) or non-symbiotic (L− *white bars*) with the fungal endophyte *E. occultans* or containing no litter (L0 *grey bars*), and placed on plots sown with seeds of *L. multiflorum,* symbiotic (E+) or non-symbiotic (E−) with the fungal endophyte *E. occultans.* Values are means and standard error ($n = 8$). Different letters indicate significant differences between treatments for each variable ($p < 0.05$)

Discussion

We present the first experimental evidence that systemic fungal endophytes contribute to disease avoidance through indirect interactions with pathogen's vectors. In this study, excluding the floral visitors reduced ergot incidence only in non-symbiotic plants taking them to the infection level of symbiotic plants, which is consistent with the hypothesis of endophyte-induced changes in the VOCs which play a role in determining their behavior and reducing their ability to spread the disease. While no differences were detected in sclerotia survival under E+ or E− plants and their litter, our data show that endophyte presence may perform a variety of defensive functions beyond direct effects, through indirect interactions with intermediary flower visitors, an ecological mechanism that contributes to the current explanation of symbiosis protection against natural enemies.

Our experimental design allowed us to detect that the association of *L. multiflorum* with *Epichloë occultans* reduced the likelihood of grass flowers to be reached by *Claviceps purpurea,* through infection-related mechanisms. Even though host grasses are mostly wind pollinated, the protection observed in symbiotic plots could be due to a negative effect of endophytes on spore vectors rather than

Table 1 Proportion of organisms (number of individuals per gram of dry soil/total number) extracted from soil samples collected from plots sown with seeds of *L. multiflorum* in the presence (E+) and absence (E−) of fungal endophyte *E. occultans* ($n = 8$)

	Collembola Mean (SE)	Acari Mean (SE)	Larva insecta Mean (SE)	Formicidae Mean (SE)
E+	0.52 (0.1)	0.38 (0.07)	0.04 (0.03)	0.06 (0.06)
E−	0.36 (0.11)	0.46 (0.09)	0.12 (0.04)	0.05 (0.03)

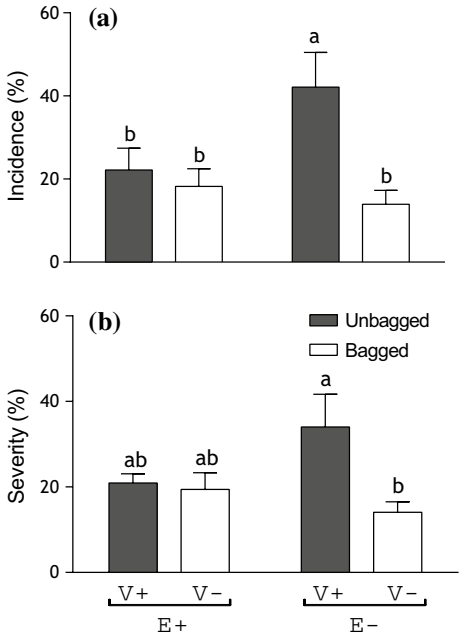

Fig. 3 Effects of endophytic fungi on **a** incidence (% infected spikes) and **b** severity (% seeds replaced by sclerotia) in *L. multiflorum* plants symbiotic (E+) and non-symbiotic (E−) with the endophyte fungus *E. occultans,* within unbagged (V+ *grey bars*) or bagged (V− *white bars*) spikes in order to avoid the contact with vectors of *C. purpurea.* Values are means and standard error ($n = 6$). *Different letters* indicate significant differences between treatments for each variable ($p < 0.05$)

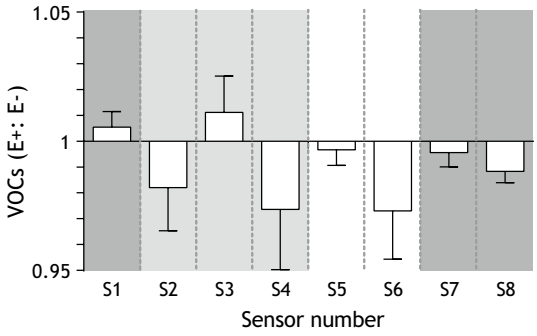

Fig. 4 Pattern of emission of volatile organic compounds (VOCs) characterized with electronic nose (AgriNose, CNEA, Buenos Aires, AR). The electronic nose consists of an array of 8 sensors (S1–S8) and a central processor that compares the response of each sensor with a reference value. Values are the means +1 SE ($n = 8$) of the ratio between symbiotic (E+) and non-symbiotic (E−) plots. Different levels of shading indicate statistical difference in the emission for a given sensor between symbiotic and non-symbiotic plants (*dark grey* $p < 0.05$; *light grey* $0.05 < p < 0.06$; *white* $p > 0.06$)

a direct impact on pathogen growth or host resistance once the spore has settled in the flowers. The difference in disease infection between vector exclusion levels in non-symbiotic plots provides evidence of the pathogen active dispersal, assuming that only abiotic factors (i.e., wind, water) spread pathogen spores from infected flowers to healthy ones across bagged spikes. Although the identity of the vectors is unknown, census performed in the same sites revealed that 16 species from 8 different families visited and foraged *L. multiflorum* spikes (Table S1, Supplementary material). Most of the insects captured exclusively consume nectar and could be playing a role in the dispersal of *C. purpurea,* In particular, *Polybia scutellaris,* which showed the highest abundance, has been reported to feed on honeydew and thus disperse conidia of *C. purpurea* in the Pampas region (Telleria 1996). Moreover, experimental studies performed in this same area revealed that insects previously described as vectors of *C. purpurea* are abundant and play the role of pathogen dispersers (Marrero et al. 2014). Honeydew production during the infection process by *C. purpurea* is of great attraction to insects, which, after feeding, carry pathogen conidia and may deposit them on healthy flowers during foraging activities (Alderman 1993; Prom and Isakeit 2003; Prom and Lopez 2004; Prom et al. 2005). An alternative activity which can contribute to pathogen dispersal by vectors is the flying between 'rest stops', a very common behavior in some *Muscidae* species.

Our results suggest that the susceptibility of *L. multiflorum* plants to disease may be regulated by endopyte presence via induced changes in production of plant VOCs. Identifying the volatile organic compounds emitted by the plants with different endophyte infection levels is outside the scope of this study and it is an important target for future research. Endophyte-induced changes in VOC emissions were recently observed for different grass species (García Parisi et al. 2014; Li et al. 2014). In particular, García Parisi et al. (2014) suggested that the effect of E. occultans on the pattern of volatile organic compounds emitted by *L. multiflorum* plants could account for endophyte protection against insect herbivores on neighboring *Trifolium repens* plants. Thus, this associational protection could also operate on herbivores or other insects transporting the pathogen to other plants that do not establish this kind of symbiosis. Endophyte-symbiotic plants have positive associational consequences on neighboring insect-pollinated plants through repelling arthropod herbivores and pathogen dispersers.

Considering this fact, a question remains about the net outcome for the neighboring plant if on the one hand vectors are reduced but they are also visited by fewer beneficial pollinators.

Regarding pre-infection mechanisms, several hypotheses emerge in order to understand the absence of differences in terms of sclerotia recovery or viability under our experimental conditions. Although several articles have found that symbioses with endophytes promote many changes in soil mesofauna (Elmi et al. 2000; West et al. 1988), our results showed no effect of the endophyte on soil community. Comparing the values obtained for individuals of mesofauna per gram of dry soil with those reported in previous works, we found fewer organisms (Lemons et al. 2005; Omacini et al. 2007). Additionally, methodological differences, such as litter mesh size or the effectiveness of the solarization treatment applied to the soil prior to the establishment of the experiment could have influenced the results (Gill and McSorley 2010). The time of the year in which this experiment took place may also be a factor to consider in further experiments. This study only comprises the period between early autumn and late spring, which means the summer season was not included in the experiment. It is in summer when soil activity may be higher than compared to the rest of the year (Bardgett et al. 1999; Birgander et al. 2014; Waldrop and Firestone 2006).

In conclusion, we demonstrated that the symbiosis between *L. multiflorum* and *E. occultans* can promote host plant escape from *C. purpurea* infection through the deterrence of pathogen vectors. This is an alternative mechanism to those so far proposed in other works to explain the endophyte protective role (i.e., induced resistance of host defences or fungistatic compound production) (Wäli et al. 2006; West and Gwinn 1993; Yue et al. 2000). Therefore, this study contributes to the knowledge of the complex symbiosis-mediated interactions operating in the observed reduction of pathogen infection in symbiotic grasses and many other situations in which this kind of mechanism was not previously considered.

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Author contribution statement LIP, MO and PEG conceived and designed the experiments. LIP, AGA and HJM performed the experiments. LIP and AGA analyzed the data. LIP wrote the manuscript and all authors provided editorial advice.

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