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# *Epichloid* endophytes confer resistance to the smut *Ustilago bullata* in the wild grass *Bromus auleticus* (Trin.)

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## Abstract

In this work it was studied for the first time whether asexual *Epichloë* (*Neotyphodium*) endophytes of *Bromus auleticus*, protect their host plants against the pathogenic fungus *Ustilago bullata.*
Seeds of two different ecotypes of *B. auleticus*, one of them infected with the endophyte *Neotyphodium pampeanum* (NpE+) and the other infected with the endophyte *N. tembladerae* (NtE+) and their respectively endophyte-free (NpE-/NtE-) counterparts were used. Seeds of each ecotype and endophytic status were superficially disinfected and were randomly assigned to different treatments named: S+ (smut fungus inoculated) and S- (mock-inoculated). It was evaluated the effect of *Ustilago bullata* infection on plant characteristics in every stage of their life cycle: seedling emergence, vegetative growth, mortality and smut symptoms in the florets.
In NtE+ infected plants, smut disease was almost completely suppressed, whereas in their endophyte-free counterparts (NpE-) the incidence of smut symptoms reached 64%. In NpE+ infected plants smut incidence was significantly lower (7%) than in endophyte-free plants (39%). Although *U. bullata* infection decreased the emergence rate of both endophyte-infected and endophyte-free plants, neutral or protective effects of the

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endophytes were observed in seedling development and survival. The survival during the first year of NtE+ plants was higher than in their NtE- counterparts.
These results indicate a strong beneficial effect of vertically transmitted endophytes against this pathogen.

**Keywords**

Defensive mutualism; Grass-endophytes; *Neotyphodium*; Smut disease; *Ustilago bullata*

## 1. Introduction

Some cool-season grasses (subfamily Pooideae) establish symbiotic associations with endophytic fungi of the genus *Epichloë* Tul. and their asexual derivatives *Neotyphodium* Glenn, Hanlin & Bacon (Clavicipictaceae, Hypocreales, Ascomycota). This association is quite specific and so each endophytic species is able to colonize one or a few host species. These fungi colonize the plant shoot meristems where they grow systemically in the apoplast of developing leafs and culms obtaining nutrients (Kuldau and Bacon, 2008). Since its growth is synchronized with the growth of the host plant and does not require the degradation of cell walls of the host, no noticeable symptoms of endophytic infection are produced (Christensen et al., 2008; Christensen and Voisey, 2007). *Epichloë* species produce stromata with perithecia in the culms of reproductive tillers avoiding the development of the flowers, causing total or partial sterility of the host plant (*choke disease*). Ascospores produced in the perithecia are forcibly discharged and are responsible for the infection of new plants. Some *Epichloë* species and most of *Neotyphodium* species do not produce stromata. In these asexual species, hyphae colonize meristems of the developing flowers and remain visible, in the mature seeds, between the aleurone cell layer and the seed coat (Schardl et al., 2004; White, 1993). Thus, these endophytes are vertically transmitted through the seeds of the host plant.
The associations between grasses and epichloid endophytes, mainly those established with vertically transmitted endophytes, are considered in general as mutualists (Clay and Schardl, 2002; Müller and Krauss, 2005; Schardl et al., 2004). The plant provides photosynthates and shelter to the endophytes and they provide several benefits to the host

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plant. Among these benefits, the most important are protection against herbivores, mediated by the production of different fungal alkaloids including loline and peramine, mainly toxic to insects, and lolitrems and ergot alkaloids that affect primarily cattle (Bacon, 1977; Clay and Schardl, 2002; Lane et al., 2000; Latch, 1993; Panaccione et al., 2006; Popay et al., 2009; Schardl et al., 2007; 2004; Schardl and Phillips, 1997; Torres et al., 2008). Increased growth and drought resistance have also been attributed to these endophytes in agronomic and native wild grasses (Clay, 1987; Iannone and Cabral, 2006; Novas et al., 2003). However, the endophyte may be detrimental under some environmental conditions and in some host species (Cheplick and Faeth, 2009; Faeth et al., 2004).

Endophytes seem to protect their host against some fungal pathogens (Bonos et al., 2005; Clarke et al., 2006; Gwinn and Gavin, 1992; Nan and Li, 2000; Yue et al., 2000) and also to modulate positively or negatively the interaction between their hosts and arbuscular mycorrhizal fungi (AM) (Chu-Chou et al., 1992; Guo et al., 1992; Liu et al., 2011; Mack and Rudgers, 2008; Müller, 2003; Novas et al., 2005; 2009; Omacini et al., 2006).

Smut fungi (Ustilaginales, Basidiomycota) are common pathogens of cereals and are studied because of their impact on agriculture worldwide (Agrios, 2005; Wilcoxson et al., 1996). These pathogens cause diseases and losses in crops (Martínez-Espinoza et al., 2002; Wilcoxson et al., 1996) and also infect wild grasses, such as *Festuca* and *Lolium* (Durán and Fischer, 1961; Vánky, 1994).

Although several smut fungi species may present differences in their life cycles, all of them cause sterility in their hosts. The ovary of the infected plants is replaced by the pathogen that produces masses of spores, known as teliospores, in the sori within host tissues (Martínez-Espinoza et al., 2002). Teliospores are resting spores that are spread by wind and remain in the soil or attached to the lemma and palea or to the cariopses coat (Agrios, 2005). Dikariotic teliospores that undergo karyogamy, germinate along with the seed forming a germ tube (promycelia) (Alexopoulos et al., 1996; Meyer et al., 2001). The diploid nucleus migrates to the promycelium and undergoes meiosis forming four haploid basidiospores. Basidiospores can either unite as compatible mating types producing the infection hypha, or they can proliferate mitotically to produce sporidia. Sporidia of compatible mating types may then fuse to penetrate the host as a dikaryotic hypha (Agrios, 2005).



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*Ustilago bullata,* the causal organism of head smut of grasses, is a highly polymorphic and systemic smut fungus that infects its host soon after the emergence of the coleoptile from the seed (Falloon, 1979; Fischer, 1940). The presence of the fungus in their host becomes apparent at anthesis when the glumes and ovary of infected hosts are destroyed, being replaced by a dark black mass of teliospores (Falloon and Hume, 1988). The effects of *U. bullata* on *Bromus* spp, invasive species in USA, or forage species have been extensively studied by Falloon (1976; 1979); Falloon and Hume (1988); García-Guzmán et al. (1996); Hirschhorn (1986); Meyer et al. (2001).

*Bromus auleticus* Trin., is a native perennial grass that inhabits grasslands of Argentina, Uruguay and southern Brazil. In Argentina, *B. auleticus* is infected by two species of endophytes with a frequency of infection higher than 95% in most of the studied populations (Iannone et al., 2009). This grass has been reported as host of the smut *U. bullata* Berk (Astiz Gassó and Molina, 2010; Traverso, 2001). Field surveys carried out in Argentina indicate that, infection of *B. auleticus* by *U. bullata* has not been very commonly observed in nature, but in field assays, studying endophyte-free plants, smut symptoms produced by *U. bullata* are usually observed (De Battista, personal communication).

In grasses infected simultaneously by vertically transmitted epichloid endophytes and smut fungi, both fungi compete for the colonization of the ovary and require, in a different way, the flower for their reproduction and dissemination. If the endophyte is able to avoid the replacement of the ovary by the smut fungus, leading to the development of a normal seed, both the host plant and the endophyte will be able to reproduce and disperse.

The triple interaction host plant-epichloid endophyte-smut fungus represents an interesting model to study the effect of endophyte on pathogenic fungi that remains to be explored. Thus, the aim of this study was to establish whether vertically transmitted endophyte species confer resistance to the smut fungus in the pathosystem *Ustilago bullata-Bromus auleticus* Trin.

## 2. Materials and methods

### *2.1. Plant and smut fungus material*

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Endophyte infected (E+) and endophyte-free (E-) seeds of two different ecotypes of *B. auleticus*, originally from Intendente Alvear, La Pampa province (LP ecotype), Argentina, infected with *Neotyphodium pampeanum* Iannone & Cabral and from El Palmar (EP ecotype), Entre Ríos province, Argentina associated with *Neotyphodium tembladerae* Cabral & White (Iannone et al., 2009) were used. Endophyte-free seeds of each ecotype were obtained in 2007 by loss of endophyte viability in long term stored seeds. Since 2007, E+ and E- plants of each ecotype were grown in the field and seeds are collected every year. Seeds used for all the experiments described below were collected during December from the previous year to each experiment described below.

Teliospores of *U. bullata* were collected from infected *Bromus catharticus* plants in December 2008 and 2009. Diseased florets exhibiting fully ripen sori were collected and mildly ground in a mortar and a pestle to release the teliospores. The powder containing teliospores and pieces of vegetal tissues was sieved in a 1mm sieve to remove plant tissues. Teliospores were kept dry at 4 °C and were used during the first 12 months after the collection. For the taxonomic identification of *Ustilago bullata*, ITS region was amplified by PCR accordingly to White et al. 1990. PCR product was purified and sequenced in an ABI 3730xl DNA Analyzer. Identification of the smut fungus was performed by means of BLAST on the GenBank database and followed by phylogenetic analyses using Maximum Parsimony (Winclada v0.9.9) (Nixon, 1999) and MrBayes algorithms (Mr. Bayes 3.2) (Ronquist et al., 2012) (not shown).

*2.2. Endophyte detection*

The endophytic status of the seed lots and plants was established by the examination of the endophyte in seeds previous to each experiment and in the seedlings or plants at the end of each experiment. To confirm the presence of the endophyte in seeds, caryopses were soaked for 5 h in a 10 % v/v aqueous solution of sodium hydroxide at room temperature (22–24°C), and then rinsed and stained with aniline blue (0.1% aqueous) (Clark et al., 1983). Endophytic mycelia were visualized in parenchymal tissues within the culm pith or in the parenchyma of peeled sheaths, aniline blue stained as mentioned and observed under a light microscope. Plants were considered as endophyte infected if a mass of dark blue

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hyphae was observed between the aleurone cell layer and the seed coat or when characteristic unbranched hyphae were observed in parenchymal tissues.

*2.3. Treatments*

For all the experiments discussed below, seeds of each ecotype and endophytic status (*N. pampeanum*-infected (NpE+); *N. pampeanum*-free (NpE-); *N. tembladerae*-infected (NtE+) and *N. tembladerae*-free (NtE-)) were superficially disinfected by consecutive washes as follows: ethanol 50%, 1 minute; sodium hypochlorite 2%, 5 minutes and ethanol 50%, 1 minute. E+ and E- seeds of each ecotype were randomly assigned to the different treatments named: S+ (smut fungus inoculated) and S- (mock-inoculated). To achieve this, seeds assigned to S+ treatments were placed in Petri dishes and a powder of teliospores (0.15 mg teliospores.seed$^{-1}$) was poured on them. For control treatments, a mock-inoculation with heat inactivated teliospores (180ºC for 4 hours) was done. The Petri dishes were closed and gently shaken for 5 minutes to obtain a homogeneous spore distribution on the seeds. In this way, 4 treatments were established for each ecotype named as follows: NpE+S+; NpE+S-; NpE-S+;NpE-S-; NtE+S+; NtE+S-; NtE-S+ and NtE-S-. Before the inoculation, the viability of the teliospores was evaluated by preparing a suspension of teliospores in water (1.5×10$^{8}$ spores.ml$^{-1}$). Fifty µl of the solution were spread in Petri dishes with water agar 2% and incubated 6 hours in darkness at 24°C. Spores able to germinate (producing a germinating tube) were considered as viable and the percentage of germination was registered. Teliospores viability ranged between 30 and 60%, and in those inactivated for the S- treatments the percentage of germination was zero (even when the inactivated teliospores were re-checked after 72 hours of incubation).

*2.4. Effect of Ustilago bullata on seedling emergence and plant development*

In order to determine the effect of infection by *U. bullata* on *B. auleticus* seedling emergence and development, 150 seeds of each treatment and ecotype were sown in ten Petri dishes (ten replicates with fifteen seeds/dish in each treatment) filled with sterilized sand and incubated in a growing chamber at 22 °C under 12 hours photoperiod. The percentage of seed germination was recorded and shoot length was measured after 15 days

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from the sowing. Results were analyzed by a two way ANOVA ($p<0.05$) for each ecotype where the inoculation with the smut fungus and the endophytic status were the main factors. All data analyses were performed using the Infostat software (Di Rienzo et al., 2011).

*2.5. Evaluation of plant survival and smut symptoms development*

One hundred and fifty seeds of each ecotype and endophytic status were inoculated with teliospores as described above. Seeds of each treatment were germinated in trays filled with sterilized sand in a growth chamber at 22 °C under 12 hours photoperiod. Two-month-old seedlings were transplanted individually to 25 cm deep x 15 cm in diameter pots, filled with commercial garden soil: sand: perlite 3:1:1 and transferred outdoors to the experimental field of the Facultad de Ciencias Exactas y Naturales, University of Buenos Aires where they were allowed to grow and produce flowers. During this period, the survival of the two-month-old seedlings (before being transplanted to pots in the field), plant survival before flowering and the incidence of the disease in NpE+/NtE+ or NpE-/NtE- plants were evaluated. The incidence of the disease in each treatment was evaluated as the number of plants with symptoms (flowers with sori/number of flowered plants). For each ecotype, the differences among treatments in seedling and plant survival and disease incidence were compared by means of a *Chi-square* test of homogeneity of proportions and the Marascuilo procedure was used to make comparisons between all pairs of groups (Marascuilo and McSweeney, 1977).

*2.6. Vertical transmission of the endophyte via seeds*

In those plants that produced seeds, the transmission of the endophyte was evaluated by checking the presence of endophyte in the seeds, as previously described.

## 3. Results

*3.1. Seedling emergence*



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The inoculation with teliospores of *U. bullata* in seeds decreased the overall percentage of emergence of *B. auleticus* (in LP ecotype $F_{1;36}$=63.46 $P$<0.0001 and in EP $F_{1;36}$=5.28 $P$=0.0275). The presence of the endophyte did not affect seedling emergence (LP: $F_{1;36}$=0.40 $P$=0.5293; EP: $F_{1;36}$=2.98 $P$=0.0930) (Fig.1).

In LP ecotype the seedling emergence in NpE+S+ treatment was 49% lower than in NpE+S- treatment, whereas in NpE-S+ treatment was 35% lower than in NpE-S- treatment, but the difference in the emergence between NpE+S+ and NpE-S- treatment was not statistically significant (Fig. 1A). In EP ecotype seedling emergence decreased 16% in NtE+ seeds while in NtE- seeds the germination was 23% lower than in the control (Fig. 1B).

*3.2. Seedling growth*

No significant differences were observed in the shoot length between E+ and E- plants of each ecotype (LP: $F_{1;36}$=1.31, $P$=0.2592; EP: $F_{1;36}$=2.43, $P$=0.1278) (Fig. 2). However, in both ecotypes, seedlings were negatively affected by the presence of the smut fungus (LP: $F_{1;36}$=138.14, $P$<0.0001; EP: $F_{1;36}$=39.46, $P$<0.0001). *Ustilago bullata* effects were more evident in LP ecotype where NpE+S+ and NpE-S+ plants were 46% and 43% smaller respectively than their S- counterparts (Fig. 2A). In EP ecotype, NtE-S+ seedlings were 39% smaller than the NtE-S- ones, whereas NtE+S+ seedlings were only 29% smaller than their NtE+S- counterparts (Fig. 2B), but this difference was not statistically significant.

*3.3. Plant survival*

The inoculation of seeds with teliospores of *U. bullata* decreased the seedlings survival of both ecotypes, during the first 60 days of growth (LP: $\chi^2_{0.95;3}$=32.02; $P$<0.0001and EP: $\chi^2_{0.95;3}$=61.04; $P$<0.0001) (Fig. 3A and B). However, while in LP ecotype no differences were observed due to the endophytic status among the smut inoculated plants, in NtE+S+ seedlings was significantly higher than in the NtE- ones.

Among the plants grown to evaluate the development of the disease at the flowering time, the percentage of survival during the first year of growth in the field was significantly

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higher in plants grown from S- seeds (Fig. 3C and D) (LP: $\chi^2_{0.95;3}$ =74.67; $P$<0.0001 and EP: $\chi^2_{0.95;3}$=78.32; $P$<0.0001).

In LP ecotype, even though only the 15% of the NpE-S+ plants survived, this value was not significantly different from the 31% of survival presented by the NpE+S+ ones (Fig. 3C). On the other hand in EP ecotype the 65.8 % of survival presented by the NtE+S+ plants was significantly higher than that observed in the NtE-S+, where only the 3% of the plants survived (Fig. 3D).

*3.4. Development of smut symptoms in field*

The presence of smut disease symptoms in the florets was evaluated in one or two year old plants grown in pots at field conditions. Disease incidence was almost totally suppressed or significantly diminished in E+ plants of both ecotypes (LP: $\chi^2_{0.95;1}$=12.67; $P$=0.0004 and EP: $\chi^2_{0.95;1}$=78.21; $P$<0.0001) (Fig. 4). None of the control plants (mock-inoculated) presented symptoms of disease (not shown in figure 4). In the plants that presented smut symptoms all the flowers were destroyed by the pathogen.

*3.5. Vertical transmission of the endophyte*

None of the NpE+ or NpE- smut-symptomless plants (from LP ecotype) produce fully ripen seeds. In EP ecotype 11 plants produced fully ripen seeds, but only two to five seeds were produced by each plant. The analysis of the presence of the endophytes in the seeds showed that all the seeds were endophyte infected; indicating that in EP ecotype, the inoculation with *Ustilago bullata* did not affected the transmission of the endophyte to the seeds.

## 4. Discussion

The present work, to our knowledge, is the first report of protective effect of *Neotyphodium* endophytes against a systemic pathogen like *U. bullata* that produces castration of the

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plants. Our findings suggest that plants of *Bromus auleticus* associated with *Neotyphodium tembladerae* or *N. pampeanum* were more resistant to the "head smut" of grasses produced by *Ustilago bullata* than endophyte-free plants.

In this work we found that, whereas in endophyte-free plants the incidence of the disease reached 39 to 64%, in endophyte-infected plants disease incidence ranged from 1 to 7%. In those plants that presented smut symptoms seed production was totally suppressed producing sterility in the affected plants. Thus, our results show that the endophytes prevent castration of the host plant, ensuring sexual reproduction of the host. Although the amount of fully ripen seeds produced by control or symptomless plants, in the S+ treatment, and checked for endophyte infection, was not enough to evaluate accurately the efficiency of the transmission of the endophyte through the seeds; our results also showed that the vertical transmission of the endophyte is not affected by the inoculation of the smut fungus. *In vitro* assays, performed in our laboratory, showed that teliospore germination is inhibited by *N. pampeanum* and *N. tembladerae* (Iannone et al., 2012b). Protective effects of epichloid endophytes against plant fungal pathogens such as *Laetisaria fuciformis* (Bonos et al., 2005), *Alternaria alternata, Fusarium* (Nan and Li, 2000), *Cercospora*, *Cryphonectria parasitica* (*in vitro*) (Yue et al., 2000), *Sclerotinia homeocarpa* (Clarke et al., 2006), *Rhizoctonia zeae* (Gwinn and Gavin, 1992) have been also reported. All together, these results are in agreement with the hypothesis of the defensive mutualism suggested for the grass-endophyte associations (Clay, 1988; 1989; Saikkonen et al., 2010).

In spite of the beneficial effects observed in E+ plants with respect to prevention of smut disease development, the endophytes had neutral effects on seedling emergence and growth, since these variables where similarly (negatively) affected by the presence of the smut fungus both in the E+ as in the E- treatments. Considering that *U. bullata* requires flower production for its dissemination, negative effects on plant survival and development should not be expected. However, these kind of effects produced by this pathogen on its host plants were also reported in *Bromus catharticus* (Falloon, 1976; García-Guzmán et al., 1996). In addition, we consider that the amount of teliospores used in each experiment was significantly higher than that expected to be found in nature since after the inoculation the seeds remained totally covered by a black coat of spores. Thus, detrimental effects of the

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smut fungus could have been enhanced and some of the protective effects of the endophyte could have been masked in our experiments. Protective effects of the endophyte could be even more important in natural conditions where the charge (inoculum) of teliospores is expected to be lower.

Different behaviors were observed between plants of different ecotypes, whereas smut development was almost totally suppressed in NtE+ plants (EP ecotype; *N. tembladerae*-infected), in NpE+ plants (LP ecotype; *N. pampeanum*-infected) the incidence of the disease was diminished but not so drastically as in NtE+ plants. In the presence of the pathogen, survival of NtE+ plants was higher than in the NtE- ones, but no differences were observed between NpE+ and NpE- plants. These differences observed in plant survival and disease incidence between the E+ plants of each ecotype seem to indicate that the protective effects of *N. tembladerae* against this pathogen are stronger than those conferred by *N. pampeanum*. However, we cannot discard that the observed differences could be due to differences in the susceptibility of each plant ecotype. Supporting our hypothesis of a higher protective capacity of *N. tembladerae*, there are *in vitro* studies that showed that *N. tembladerae* presented the highest inhibitory capacity against several fungal plant pathogens with respect to other *Epichloë*/*Neotyphodium* species (Yue et al., 2000) and against *U. bullata* (Iannone et al., 2012b).

The protective effects shown in the E+ plants against the head smut of grasses disease could be due to, 1) the endophytes preventing the infection by *U. bullata* at seedling stage or 2) the endophytes preventing the colonization of the ovary by the pathogen. The detrimental effects of smut fungus inoculation observed on seedling emergence, seedling survival and development in E+ and E- treatments would support hypothesis 2, indicating that the smut fungus is able to infect the seedlings of *B. auleticus* irrespectively of their endophytic status.

The higher survival and resistance of endophyte-infected plants to *U. bullata*, in addition to other beneficial properties observed in endophyte infected plants (Iannone et al., 2012a) could explain the higher incidence of endophytes in populations (smut-symptomless) of this host in nature. Endophyte infected plants produced more seeds than E- ones (Iannone et al., 2012a) and seed production was suppressed in E- plants when infected with *U. bullata*. However, considering that *B. auleticus* is a highly perennial plant, more long term studies

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are necessary in order to evaluate the importance of *Ustilago bullata* and the effects of both symbionts on the dynamics of the populations of this host.

Although more research should be done in order to establish the mechanism through which both fungal symbionts interact in the host plant so that the incidence of the disease is lower in E+ plants; our findings are relevant for a better understanding of the biology of the grass-endophyte symbiosis and could be also applied in grass breeding programs. Currently, studies are being conducted in our laboratory in order to evaluate the effect of the endophyte-smut fungus interactions in plant competition, and seed production in field. Finally, O'Hanlon et al. (2012) stated that more attention should be paid to dissecting the potential of fungal endophytes as biological control agents against cereal pathogens. In this sense, our studies and results on smut resistance should be expanded to other endophyte-infected grasses, mainly wild barley species as *Hordeum bogdanii*, *H. brevisubulatum* and *H.comosum.*

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**Fig. 1.** Effect of *Ustilago bullata* and *Neotyphodium pampeanum* (Np) (A) or *N. tembladerae* (Nt) (B) endophytic status on seedling emergence of *Bromus auleticus*. Endophyte infected (NpE+/NtE+) and endophyte free (NpE-/NtE-) seeds, inoculated (S+) or mock-inoculated (S-) with *Ustilago bullata*. Different letters indicate significant differences (*P*<0.05).

**Fig. 2.** Effect of *Ustilago bullata* and *Neotyphodium pampeanum* (Np) (A) or *N. tembladerae* (Nt) (B) on *Bromus auleticus* seedlings shoot length (cm). Endophyte infected (NpE+/NtE+) and endophyte free (NpE-/NtE-) seeds, inoculated (S+) or mock-inoculated (S-) with *Ustilago bullata*. Data are means; SE. Different letters indicate significant differences (*P*<0.05).

**Fig. 3.** Effect of *Ustilago bullata* and *Neotyphodium pampeanum* (Np) (A, C) or *N. tembladerae* (Nt) (B, D) on *Bromus auleticus* seedlings survival during the first two months of growth (A, B) and plants survival during the first year of growth under field conditions (C, D). Endophyte infected (NpE+/NtE+) and endophyte free (NpE-/NtE-) seeds, inoculated (S+) or mock-inoculated (S-) with *Ustilago bullata.* Different letters indicate significant differences (*P*<0.05).

**Fig. 4.** Percentage of *Bromus auleticus* plants inoculated with *Ustilago bullata* with smut symptoms in the florets. NpE+: *Neotyphodium pampeanum* infected; NpE-: *N. pampeanum* free; NtE+: *N. tembladerae* infected and NtE-: *N. tembladerae* free. Different letters indicate significant differences within each ecotype (*P*<0.05). The plants in those treatments inoculated with inactive teliospores (S-) did not present smut disease symptoms (not shown in the figure).

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Figure1

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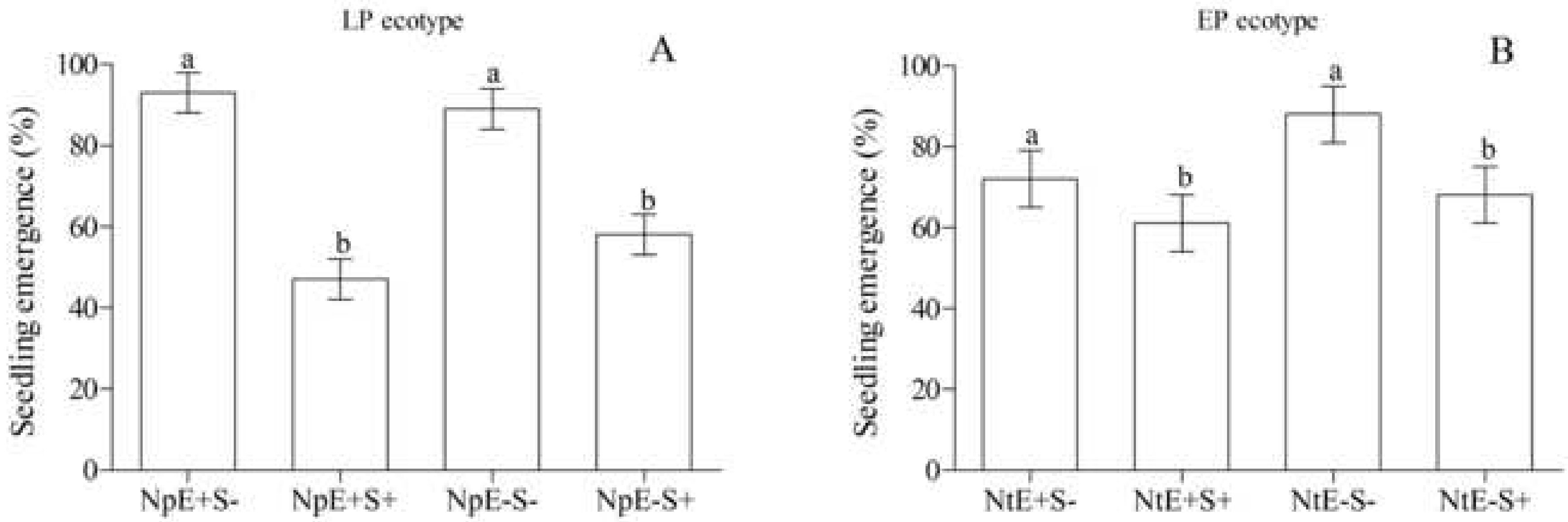


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Figure2

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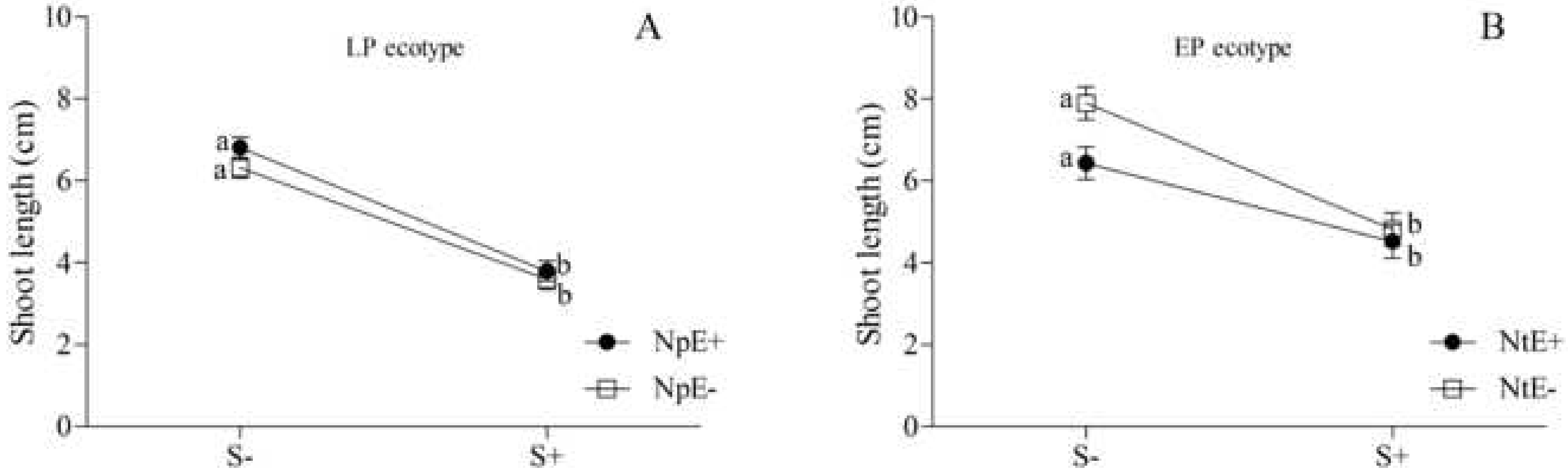


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Figure3

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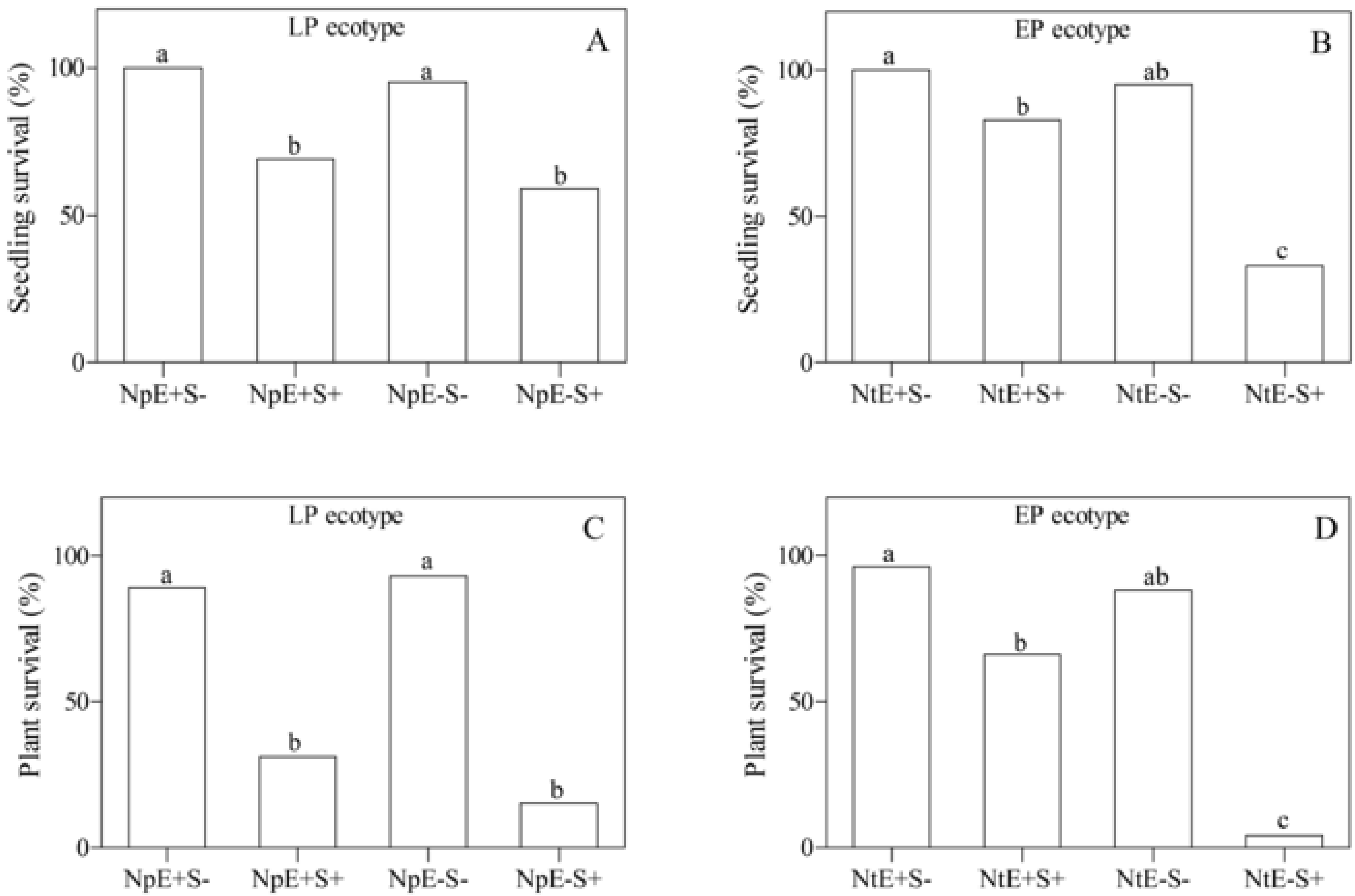


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Figure4

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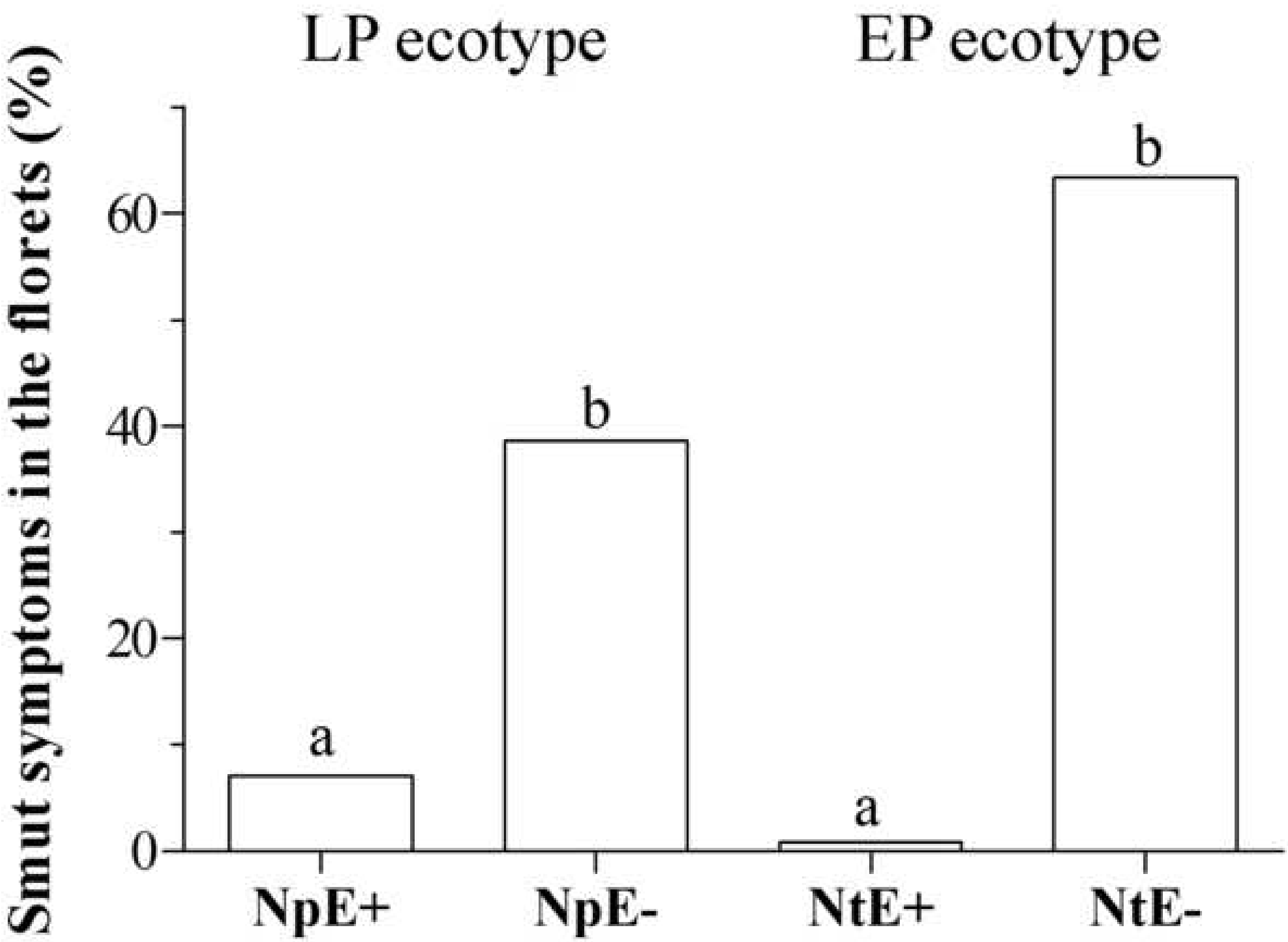

*Graphical Abstract (for review)

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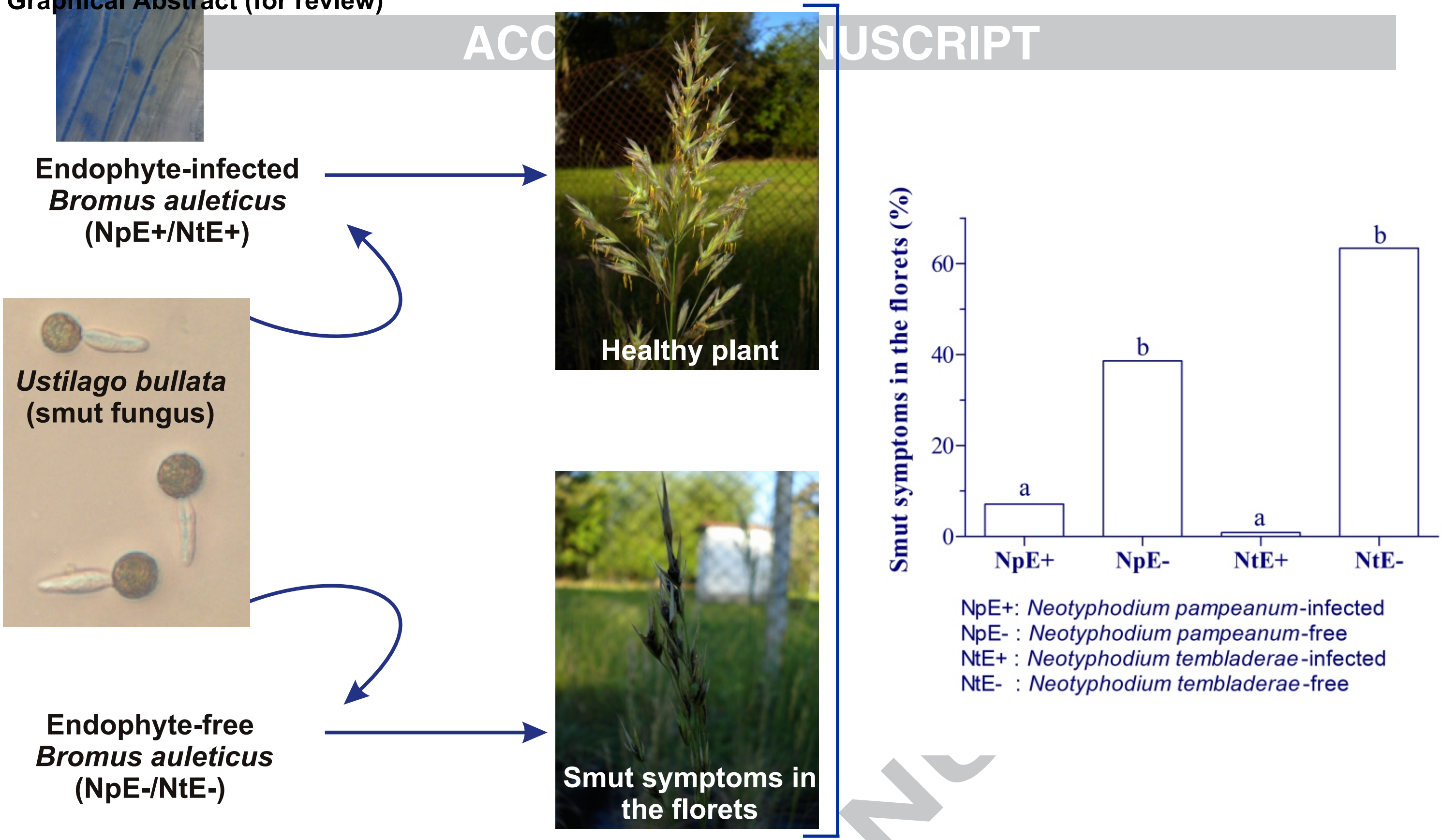

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## Highlights

Some grasses are usually co-infected by smut fungi and mutualist epichloid endophytes.

Endophytes are transmitted via seeds and smut fungi replace the seeds with teliospores.

The endophyte and the smut fungus compete in a race for the colonization of the ovary.

The effect of *Neotyphodium* spp. against head smut of grasses was evaluated.

Disease incidence was diminished in endophyte-infected *Bromus auleticus* plants.

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