

Symbiotic interactions as drivers of trade-offs in plants: effects of fungal endophytes on tall fescue

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Abstract Studying the controls on biomass allocation trade-offs in plants are important since they affect harvestable product yields and are critical to understanding symbiotic interactions. Epichloae fungal endophytes associate with cool-season grasses, growing systemically within the plant inter-cellular spaces and are transmitted through seeds. We explore the endophytes influence on the relationship between the plant reproductive and vegetative aboveground biomass (reproductive effort: RE) and on the trade-off between two components of the reproductive biomass, number and weight of panicles (RPN), using tall fescue as a model system. Naturally endophyte-colonized, manipulatively endophyte-free, and naturally endophyte-free plants from Northern European wild-populations together with the cultivar Kentucky-31 were grown under different environmental conditions (nutrients x water). The endophyte had an

effect on the RPN (E+: 6.19, ME-: 4.68 and E-: 4.40) which indicates how reproductive biomass is partitioned into number and mass of panicles, but not on RE (≈ 0.06). As expected, wild plants showed higher reproductive effort (≈ 0.06) compared to the cultivar KY-31 (0.05), irrespective of endophyte presence. Endophyte-colonized plants had lighter panicles than endophyte-free plants, a pattern that was clear among low-yielding plants. Similarly, the trade-off between RPN and RE was higher for endophyte-colonized plants. This was again evident among plants with low RE indicating that colonized plants split the yield into either greater number of panicles and/or lighter panicles. The effect of vertically transmitted endophytes has earlier been studied as ratios (e.g. RE); however, our study shows that this approach may hide size-dependent endophyte effects on these relationships. Our study reveals that *Neotyphodium* endophyte affects trade-offs in tall fescue plants in a complex manner, and is influenced by a number of biological and abiotic factors.

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Introduction

Strongly governed by trade-offs between number and mass of different organs, or ecological functions such as reproduction and survival, the relative amount of biomass allocated to different plant parts is considered a highly conserved life history trait of species (Law 1979; Niklas 1994; Weiner 2004; Reekie and Bazzaz 2005; Bonser and Aarssen 2009; Weiner et al. 2009; Poorter et al. 2012). These ratios are of interest not only for plant evolutionary ecology but also for applied sciences, as they control the biomass allocation to harvestable products (Reekie and Bazzaz 2005; Sadras 2007; Weiner et al. 2009; Poorter et al. 2012). Trade-offs between

plant functions are increasingly recognized to be critical in understanding symbiotic inter-specific interactions (Bronstein 1994; Kiers et al. 2002; Saikkonen et al. 2004; Thompson 2005; Bever et al. 2009; Zhang et al. 2011; Gundel et al. 2012).

The impact of symbiotic microorganisms on trade-offs in plants is a result of coevolutionary and ecological processes (Bronstein 1994; Kiers et al. 2002; Saikkonen et al. 2004; Thompson 2005). Nitrogen-fixing bacteria and phosphorus uptake enhancing mycorrhizal fungi are the most widely studied symbionts affecting biomass allocation of host plants (Requena et al. 1997; Thrall et al. 2011; Zhang et al. 2011). For example, absence of arbuscular mycorrhizal fungi can have comparable effects to belowground resource shortage increasing the root/shoot ratio (Zhang et al. 2011; Poorter et al. 2012). However, this effect may vary with the level of environmental resources available since mycorrhizae can also be costly to host plants under low resource availability (Johnson et al. 1997). Furthermore, ecological benefits of a particular symbiont strain may depend on the local adaptation to a specific host population under a given environmental condition which results from coevolution between partners (Requena et al. 1997; Kiers et al. 2002; Bever et al. 2009; Hoeksema 2010; Thrall et al. 2011). Systemic fungal endophytes of genus *Neotyphodium* have been found to affect plant biomass allocation modifying for example, the root/shoot ratio and reproductive effort (Hesse et al. 2003; Vila-Aiub et al. 2005; Faeth 2009). However, there are only a few studies evaluating the variation in size-dependent traits and trade-offs between fitness-related traits of host plants (Cheplick and Faeth 2009; Gundel et al. 2012).

The symbiotic interaction of grasses (Poaceae) with *Neotyphodium* fungal endophytes is highly specialized. Each fungal species is usually restricted to related grass species or genera within a tribe, with one individual strain co-habiting within the host apoplast of the aboveground tissues, passing through generations via the host seeds (i.e. vertical transmission; Clay and Schardl 2002; Saikkonen et al. 2004; Schardl 2010; but see Tadych et al. 2012). The strong stamp of mutualistic interactions is primarily due to production of fungal alkaloids that endow the host plants with an anti-herbivory mechanism (Clay and Schardl 2002; Schardl 2010). Additionally, endophytes have been found to improve persistence and growth of host plants in a wide range of environmental conditions, even though parasitic effects depressing host fitness have also been detected (Saikkonen et al. 1998a; Bouton et al. 2001; Ahlholm et al. 2002; Clay and Schardl 2002; Faeth 2002; Hesse et al. 2003; Vila-Aiub et al. 2005; Rudgers et al. 2009; Hamilton et al. 2012; Hamilton and Bauerle 2012). It has been proposed that maternally inherited symbionts may promote allocation to reproduction or more specifically, to female functions in host macroorganisms (e.g. panicles and seeds) (Ewald 1987; Saikkonen et al. 2004; Thompson 2005). Considering that the seed is the carrier of

the endophyte and the existing trade-off between seed size and number, vertically transmitted symbionts could promote seed weight or number depending on the environment (Gundel et al. 2012). An alternative pattern could be the allocation to vegetative propagation (e.g. tillers) in cross-pollinated grasses, due to the negative effect of gene flow over the natural selection pressure favoring compatible grass-endophyte combinations (Saikkonen et al. 2004; Thompson 2005; Afkhami and Rudgers 2008; Gundel et al. 2010, 2011, 2012).

Tall fescue is a stress-tolerant ruderal species allocating a high proportion of biomass to reproduction (Grime et al. 1988; Gibson and Newman 2001). However, departures from this description could be anticipated as consequences of breeding programs for forage production, geographic variation in natural selection pressures, and coevolution with fungal endophytes. Forage cultivars are bred for persistence and biomass production, grown in relatively simple vegetation communities, high nutrient environments and subjected usually, to cattle grazing or mowing (Bouton et al. 2001; Western 2001; Saikkonen et al. 2004, 2006). These human-made ecosystems contrast with natural or semi-natural grasslands which may be more complex and subjected to high plant competition and stress (Saikkonen 2000; Western 2001; Saikkonen et al. 2004, 2006). If improvement for the agronomic traits during breeding implies deterioration of other traits, differences in trade-offs among adaptive plant traits between human-managed and semi-natural conditions are expected (Denison et al. 2003; Gundel et al. 2013). For example, grazing and mowing could select for tillering in human-made habitats while plant competition could select for apical dominance in semi-natural wild communities. Endophyte colonized forage cultivars have been found to grow and reproduce more efficiently than uninfected individuals of the same cultivar (De Battista et al. 1990; Rice et al. 1990; Bouton et al. 2001). However, geographic variation in effects of endophytes on plant biomass allocation patterns in natural populations are less understood (Saikkonen et al. 2004; Thompson 2005; Gundel et al. 2012). Particularly remarkable is that genetic diversity of fungal endophytes associated to wild populations seems to be contrastingly higher to that one found in cultivars (Piano et al. 2005; van Zijl de Jong et al. 2008; Cheplick and Faeth 2009).

In this article, we explore the influence of endophyte colonization on the relationship between reproductive and vegetative aboveground biomass (i.e. reproductive effort) and on the trade-off between the two components of the reproductive biomass, number and weight of panicles, in tall fescue. Naturally endophyte-colonized (E+), manipulatively endophyte-free (ME-), and naturally endophyte-free (E-) plants from North European wild populations and the cultivar Kentucky-31 (KY-31) were grown under different environmental conditions. Since these fungal endophytes are vertically transmitted from mother plant to offspring, we hypothesized that they promote resource allocation to

reproductive -rather than vegetative organs- and apical dominance, thus promoting low number but heavier panicles (rather than higher numbers of panicles). We expected that (i) plants from wild populations will show higher reproductive effort than plants from the cultivar KY-31, and that differences should be greater when comparing wild E+ plants with KY-31 ME- plants; (ii) the symbiosis will promote fewer but heavier panicles in evolutionary (E+ vs. E-) and ecological terms (E+ vs. ME-). Considering that endophytes confer alkaloid-mediated resistance to grazing (Bush et al. 1997; Bouton et al. 2001) and that grazing reduces apical dominance promoting tillering (Murphy and Briske 1992; Agrawal 2000), (iii) the expected pattern in (ii) will be stronger in wild plant populations than in the cultivar KY-31, and (iv) more evident under high resource availability.

Materials and methods

Origin of plants and symbiotic status

Seeds of *Schedonorus phoenix* (Scop.) Holub. (ex. *Lolium arundinaceum*, syn. *Festuca arundinacea*) were harvested from three geographic locations around the Baltic Sea, the islands of Åland (Finland) and Gotland (Sweden), and the Swedish mainland coast (just for convenience, we will call them, Åland, Gotland and Sweden) in August 2003 (Saari et al. 2010). Mature seeds were hand-collected from 10 to 50 individual plants from approximately eight wild populations separated by several kilometers from each other in each location. Endophyte status of individual plants was determined by evaluating three seeds per plant using the staining method by Saha et al. (1988). All the populations presented more than 90 % of colonization by the fungus *Neotyphodium coenophialum* Glenn, Hanlin and Bacon (determination based on morphological characteristics of the hyphae; Saari et al. 2010). E+ and E- plant seeds were pooled within each geographic location (Åland, Gotland, and Sweden). E+ and E- KY-31 were collected from the experimental fields at the University of Kentucky, where the plants had been growing for more than 5 years under agronomic management (Dr. T. Phillips, *pers. comm.*). The very low genetic diversity of endophytes associated to cultivars should contrast with the relatively high genetic variability within fungal endophyte populations of wild grasses (Saikkonen et al. 2004, 2006; Cheplick and Faeth 2009). A batch of colonized seeds from each origin (Åland, Gotland, and Sweden) and the cultivar KY-31, were soaked in warm water (≈ 57 °C) for about 15 min to remove the endophyte. This method is effective in killing the fungus whilst still retaining viable seed (Saari et al. 2010). Thus we had three endophyte colonization statuses for each plant origin: naturally endophyte-colonized (E+), manipulatively endophyte-free (ME-), and naturally endophyte-free (E-) plants. Seeds from each combination of plant origin x endophytic status

were germinated in Petri dishes (9 mm filter paper) under greenhouse conditions (≈ 20 °C and natural photoperiod). Seven days after sowing, forty seedlings from each combination were transplanted to individual sand and peat (50/50, v/v) filled pots which were kept in the greenhouse.

Experimental setting

The experiment was established at Turku Botanical Garden, University of Turku, Finland (60°26'0"N, 22°10'19"E). The plants were transplanted to the common garden on August 2004 when they had approximately 3 tillers each. The site was tilled before transplantation. The experimental arrangement was a grid with 0.5 m between plants. The experiment consisted of 10 blocks, 4 plots within block, and 12 plants within each plot belonging to one of each combination of plant origin x endophyte status. The plant position within the plot was randomized. The experiment was fenced to prevent large herbivores from entering the area. Paths between plants were maintained free of weeds by hand-weeding and spraying a wide spectrum herbicide (Roundup®Bio). The endophyte status was checked for all the plants in 2005 by an immunoblot assay based on specific monoclonal antibodies to *Neotyphodium coenophialum* (Phytoscreen immunoblot test kit ENDO797-3, Agrinostics, Watkinsville, Georgia, USA). Additionally, the endophyte status of plants was verified by staining three seeds per plant (Saha et al. 1988). After triple-check the endophytic status of plants (using two different techniques), only four plants out of 480 presented an infection status not corresponding with the original labels. These plants were reassigned to a proper treatment based on the actual of symbiotic status. Four environmental treatments (control, water, nutrients, and water + nutrients) were randomly assigned to plots within each block. Three liters of tap water was poured three times per week during the growing season (June–August) to each plant assigned to water treatment. One deciliter of N-P-K fertilizer (Nurmen Y2, Kemira KnowHow, N-P-K/20-6-6) was applied twice during the growing season to each plant in the nutrient treatments.

The treatments were continued for 3 years: 2005, 2006, and 2007. Every year, all the aboveground plant biomass was removed by cutting it with a sickle at 10 cm above the soil surface at the end of the growing season (by September). Noteworthy is that the annual fluctuation in temperature and day length are the main driving forces determining the length of growing season in the study area. Thus, all the aboveground plant parts of herbs and grasses are withered during the harsh winter characterized by frozen temperatures, snow and short days to be re-grown from rhizome buds in spring. In 2007, plant panicles were counted and enclosed in pollination bags (PBS International) after flowering to avoid the loss of seeds. Ripe seeds were collected and weighed, and the aboveground biomass of plants

was separated by reproductive (panicles) from vegetative biomass, dried at ≈ 70 °C for 48 h in an oven and weighed.

Statistical analyses

The effects of endophyte colonization status, plant origin, environmental conditions and their interactions on the reproductive effort (i.e. the ratio between reproductive and vegetative aboveground biomass per plant) and the relative panicle number (i.e. trade-off between number and weight of panicles) were tested by mixed effect models in R (version 2.13.0; R Development Core Team 2011) with the lme function of the nlme package (Pinheiro et al. 2011). The trade-off between number and weight of panicles was evaluated through the “relative panicle number”, estimated as the ratio between panicle number and reproductive biomass. Consequently, a low relative panicle number indicates a low number of panicles per unit of reproductive biomass. This allowed us to evaluate the trade-off between panicle number and size without the confounding influence of among-plant variation in total reproductive allocation. Endophyte colonization status, plant origin and treatments of nutrients and Water, were used as fixed effects, and all the interactions (double, triple, and quadruple) were estimated as well. Random effects considered that plants were nested within plots, and plots nested within blocks using a random intercept model (p. 106 in Zuur et al. 2009), and normal distribution for the residual variation was assumed. Significance of fixed effects was tested through sequential analysis of variance (ANOVA). Relative panicle number was log₁₀ transformed to meet model assumptions.

Since analyzing biomass allocation patterns as ratios may be inadequate to reveal allometric effects (Weiner 2004; Weiner et al. 2009), we examined the relationship between the different plant parts. The relationship in biomass allocation among different plant parts was estimated as: 1) reproductive biomass as a function of vegetative aboveground biomass, 2) number of panicles as a function of reproductive biomass, and 3) relative panicle number as a function of reproductive effort (Fig. 4; [Supplementary Material](#)). In addition, each of these models also included the effects of endophyte colonization status, plant origin, nutrients and water addition, and their interaction with the quantitative predictor (e.g. vegetative aboveground biomass for the model 1). The most parsimonious model was obtained using sequential ANOVA removing complex interactions first and leaving only significant terms. Reproductive biomass, vegetative aboveground biomass, number of panicles, relative panicle number, and reproductive effort were log₁₀ transformed to express the slope of these relationships on a common scale and also to meet model assumptions (Fig. 4; [Supplementary Material](#)).

Results

Plant reproductive effort was affected by plant origin and nutrients, while it was not significantly affected by either the endophytic status or water treatment (Table 1; Figs. 1 and 3). The reproductive effort of fertilized plants was on average 43 % lower compared to unfertilized ones being 0.025, 0.042, 0.035 and 0.039 in fertilized and 0.079, 0.099, 0.074 and 0.088 in unfertilized KY-31, Åland, Gotland and Sweden plants, respectively (Fig. 1).

Reproductive biomass increased linearly with vegetative biomass (Fig. 4a, [Supplementary Material](#)). The results for the relationship between reproductive and vegetative biomass matched those observed for reproductive effort except for a significant effect of water treatment. Although both ME- and E- fitted the same regression line which was different from that of E+ plants (Fig. 4a), the positive relationship between reproductive biomass and vegetative aboveground biomass was not affected by the endophyte colonization status ([Supplementary Material](#)). In addition, reproductive biomass increased with water but decreased with nutrient addition, after having account for changes in vegetative biomass. In general, plants from KY-31 presented lower reproductive biomass than plants from the wild origins ([Supplementary Material](#)).

The relative panicle number was negatively affected by water treatment while the effect of nutrients treatment depended on its interaction with plant origin (Table 1). It was also marginally affected by the endophyte status of the plant ($P=0.055$). KY-31 plants almost doubled the relative panicle number compared to plants from the other populations in the nutrient treatment (i.e. 10.9, 6.1, 5.4, and 5.1 for KY-31, Åland, Gotland and Sweden origins, respectively) (Fig. 2). The relative panicle number of fertilized plants was 21 % higher compared to unfertilized ones but 21 % lower under water treatment than without additional water (Figs. 2 and 3). Relative panicle number across all the plant origins and environmental treatments was on average, 6.19, 4.97 and 4.40 for E+, ME- and E-, respectively.

Similarly to the relative panicle number, the minimum adequate model of the positive relationship between number of panicles and reproductive biomass (Fig. 4b) took into account the two-way interactions between reproductive biomass x endophyte colonization status, and reproductive biomass x plant origin but also, the single effect of nutrients and water ([Supplementary Material](#)). The lower and the higher interaction coefficients of reproductive biomass with E+ and ME- plants, respectively, on panicle number is clearly appreciated in the overall slopes of the linear regressions plotted in the figure (Fig. 4b). The interaction of reproductive biomass with plant origin was negative for KY-31 while positive for Gotland and Sweden in both cases relative to Åland ([Supplementary Material](#)).

Table 1 Sequential ANOVA results for the mixed effect models evaluating the effects of endophytic status (E+, ME-, and E-), plant origin (Åland, Gotland, Sweden, and KY-31), environmental treatments (nutrients and water addition), and their interactions on the reproductive effort and the relative number of panicles of *Schedonorus phoenix* plants

numDF = degrees of freedom for the numerator in the *F* test
denDF = degrees of freedom for the denominator in the *F* test

Source	numDF	Reproductive Effort			Relative panicle number		
		denDF	<i>F</i> -value	<i>p</i> -value	denDF	<i>F</i> -value	<i>p</i> -value
(intercept)	1	353	297.6	<.001	347	1308.2	<.001
Water (W)	1	27	1.3	0.263	27	7.1	0.013
Nutrient (N)	1	27	91.9	<.001	27	40.6	<.001
Origin (O)	3	353	3.9	0.010	347	11.6	<.001
Endophyte (E)	2	353	0.6	0.533	347	2.9	0.055
W x N	1	27	0.2	0.671	27	1.0	0.337
W x O	3	353	0.2	0.911	347	1.4	0.256
N x O	3	353	0.9	0.442	347	4.3	0.006
W x E	2	353	1.1	0.340	347	1.6	0.195
N x E	2	353	0.6	0.574	347	0.1	0.922
O x E	6	353	1.2	0.332	347	1.9	0.089
W x N x O	3	353	0.4	0.765	347	0.6	0.639
W x N x E	2	353	0.6	0.569	347	1.1	0.348
W x O x E	6	353	0.9	0.471	347	1.2	0.297
N x O x E	6	353	1.3	0.250	347	1.8	0.107
W x N x O x E	6	353	1.0	0.441	347	1.3	0.260

The slope of the general negative relationship between relative panicle number and reproductive effort (i.e. trade-off) (Fig. 4c) depended on the three-way interaction between reproductive effort, plant origin and nutrients addition, and also on the two-way interaction between reproductive effort x endophyte colonization status, and between reproductive effort x water addition (Supplementary Material). In general, differences between nutrient treatments and plant origins had

a major influence on the relationship between relative panicle number and reproductive effort, whereas the effect of endophyte infection status had a relative lower impact. The effect of endophyte on the negative relationship between relative panicle number and reproductive effort was significantly higher for E+ than for ME- compared to E- (Fig. 4c; Supplementary Material). Consequently, both ME- and E- plants fitted the same regression line which was different from

Fig. 1 Reproductive effort (ratio between reproductive biomass and plant aboveground biomass) of *Schedonorus phoenix* plants from the four origins (Åland, Gotland, Sweden and KY-31) in relation to plant fungal endophyte status (Naturally endophyte-colonized: E+, manipulatively endophyte-free: ME-, and naturally endophyte-free: E-) growing under two conditions (no added nutrients -, or added nutrients +). Values are means \pm SE ($n \approx 20$)

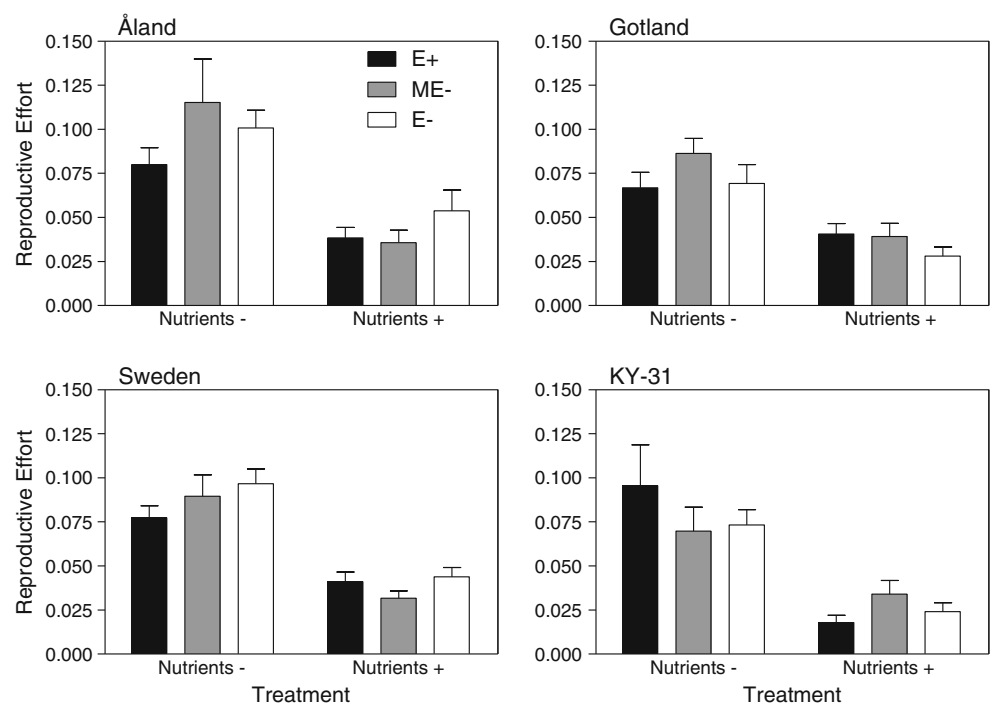
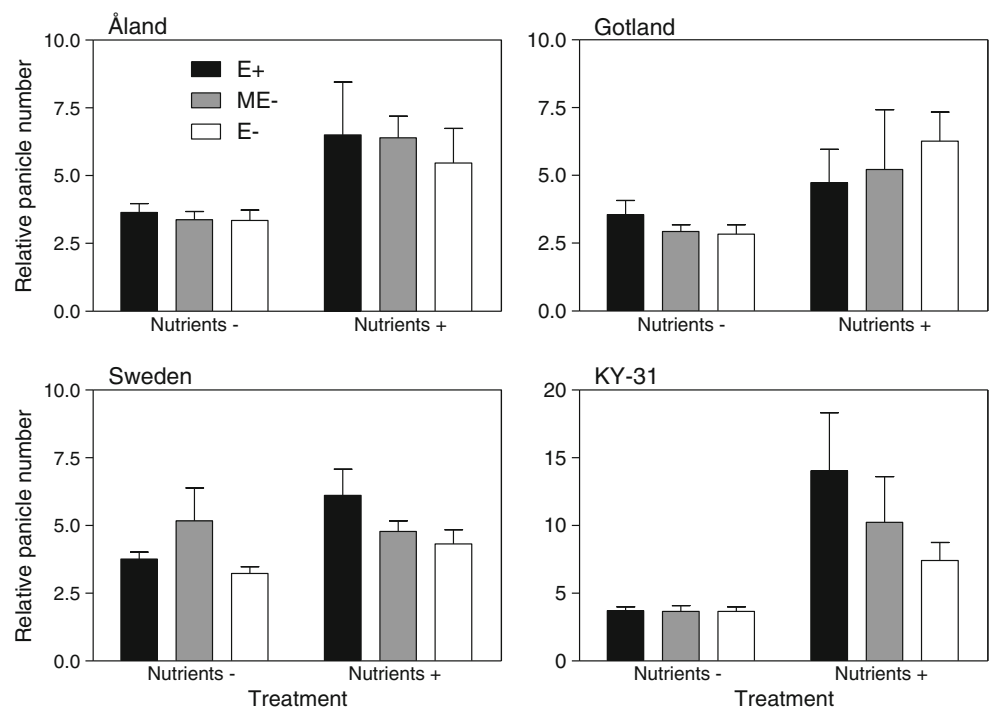


Fig. 2 Relative panicle number (ratio between panicle number and reproductive biomass) of *Schedonorus phoenix* plants from the four origins (Åland, Gotland, Sweden and KY-31) in relation to plant fungal endophyte status (Naturally endophyte-colonized: E+, manipulatively endophyte-free: ME-, and naturally endophyte-free: E-) growing with low and high nutrient availability (nutrients - and nutrients +). Values are means \pm SE ($n \approx 20$). Note that the scale of y-axis in KY-31 plot is different from the other three plots



the one of E+ plants (Fig. 4c) indicating a higher relative panicle number/reproductive effort trade-off for E+ plants than for ME- and E- plants.

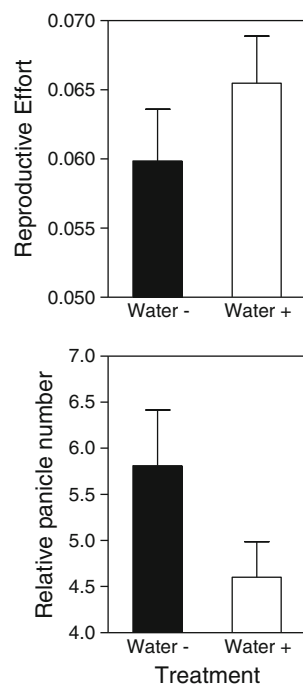


Fig. 3 Reproductive effort (ratio between reproductive biomass and plant aboveground biomass) and relative panicle number (ratio between panicle number and reproductive biomass) of *Schedonorus phoenix* plants growing with different water availability (water - and water +). Values are means \pm SE ($n \approx 140$)

Discussion

We found effects of systemic, vertically transmitted fungal endophytes on the relative panicle number (a measure of how reproductive biomass is partitioned into number and mass of panicles) but not on the ratio between reproductive and vegetative aboveground biomass (reproductive effort). As predicted, wild plants showed higher reproductive effort compared to the cultivar KY-31, but it was irrespective of the endophyte colonization. On the other hand, E+ plants had lighter panicles [6.19 panicles (#) / reproductive biomass (g)] than either E- [4.40 panicles (#) / reproductive biomass (g)] or ME- plants [4.68 panicles (#) / reproductive biomass (g)] which are in contrast to our prediction. Finally, the last two predictions which were about the differential effect of endophyte colonization among populations (iii) and treatment of nutrients (iv) remains unsupported by our results due to the lack of interacting effects between endophyte and these two factors.

Endophyte colonization had no effects on the positive relationship between reproductive biomass and vegetative aboveground biomass indicating that the allocation to reproduction in colonized and endophyte-free plants was independent of plant size. However, we were able to detect an endophyte effect on the positive relationship between number of panicles and reproductive biomass. The interactive effect of endophyte colonization with reproductive biomass on panicle number was negative. Firstly, the number of panicles of E+ plants appears to be greater than E- and ME- plants when biomass was low but the difference was

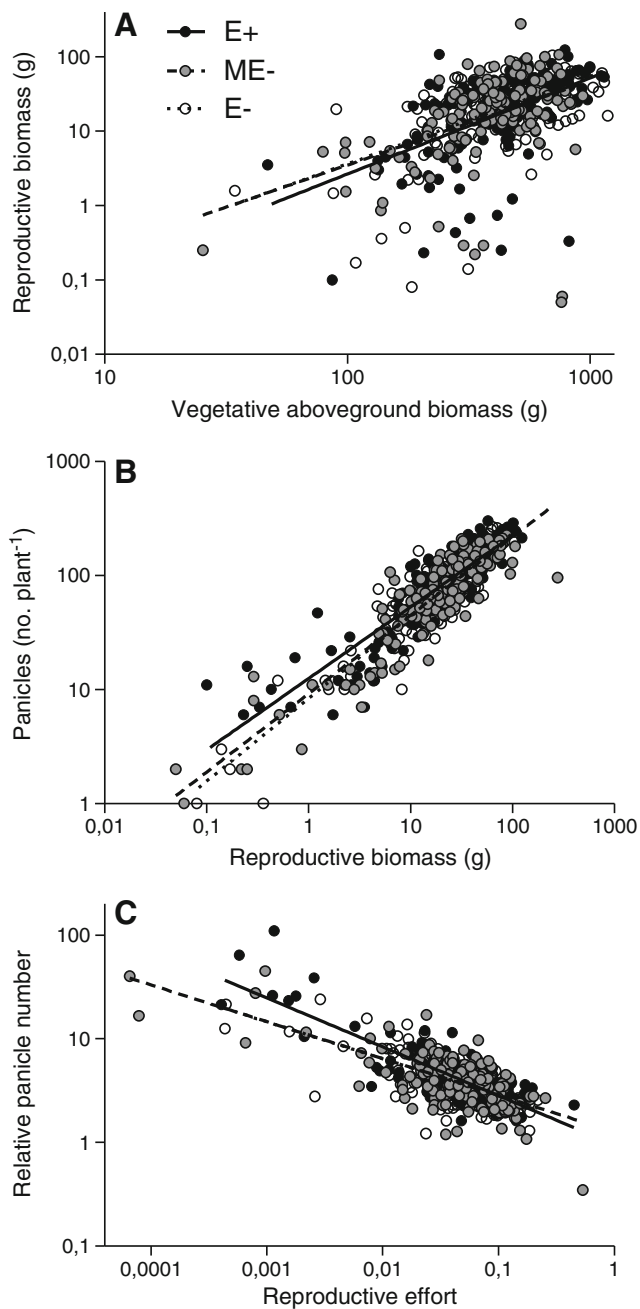


Fig. 4 Relationships between reproductive and aboveground biomass (a), panicle numbers and reproductive biomass (b), and relative panicle number and reproductive effort (c) for *Schedonorus phoenix* plants of different fungal endophyte status (Naturally endophyte-colonized: E+, manipulatively endophyte-free: ME-, and naturally endophyte-free: E-). All the plants from different origins and environmental treatments are presented (E+, $n=145$; ME-, $n=144$, and E-, $n=142$), and all axes are in Log₁₀ scale. Note that plants naturally colonized but manipulatively endophyte-free (ME-) yielded similar patterns to naturally endophyte-free plants (E-), and both differed from endophyte-colonized (E+) plants

not so in plants having higher biomass. Secondly, very low numbers of panicles were detected only in very low yielding E- plants. The absence of colonized plants with low reproductive biomass producing no panicles is likely related to

metabolic costs of the fungus for the host (Cheplick et al. 1989; Ahlholm et al. 2002; Gundel et al. 2011, 2012). Similar results have been detected also in *Lolium multiflorum* and *N. occultans*. For example, Gundel et al. (2012) detected very low reproductive biomass and seed number only in E- *L. multiflorum* plants. Therefore, the interactive effects of endophyte colonization with plant biomass can remain unrevealed when allocation patterns are studied as ratios (Weiner 2004, Weiner et al. 2009; Gundel et al. 2012).

The negative relationship between relative panicle number and reproductive effort indicates a trade-off between both partitioning functions of plant biomass. Overall, plants with higher reproductive effort showed lower relative panicle number; i.e., if more biomass was allocated to reproduction, the inflorescences were either heavier or lower in number or both. The relationship between relative panicle number and reproductive effort was affected by the endophyte colonization status. The higher negative coefficient for E+ plants relative to E- plants corresponds to a steeper slope in the negative relationship between relative panicle number and reproductive biomass. Although the overall reproductive effort of plants was unaffected by endophyte colonization, E+ plants tended to have higher relative panicle number, and only uncolonized plants (E- and ME-) produced panicles if the plant biomass remained very low. This indicates costs from endophyte colonization to the host plant (Cheplick et al. 1989; Ahlholm et al. 2002; Faeth 2002; Hesse et al. 2003; Gundel et al. 2011, 2012). In grasses, the induction of reproductive meristems is primarily controlled by the size of plant, vernalization and photoperiod while the growth of panicles and the biomass allocated to seeds is determined later during the growing season (Murphy and Briske 1992; Weiner et al. 2009). The available resources can be allocated to either a few large or many small reproductive panicles.

Plant origin strongly affected the reproductive effort and relative panicle number of plants, probably as a consequence of geographic differences in natural selection pressures, and artificial selection in the cultivar KY-31. On average, Gotland and KY-31 had similar reproductive effort (0.05) which was lower than Sweden (≈ 0.06), whereas Åland showed the highest reproductive effort (0.07). The cultivated (KY-31) origin showed consistent higher relative panicle number (7.68) than the three wild populations (4.51). These results are in agreement with previous reports suggesting that the plants from the cultivar KY-31 presented a significantly higher panicle/tiller ratio compared to plants from the wild origins although there were no differences in the total number of tillers (Gundel et al. 2013). The consistent difference is likely a result from the adaptation of European wild plants to the strong local seasonality and related environmental cues (e.g. photoperiod). In contrast, KY-31 is cultivated in high nutrient agro-ecosystems at

lower latitudes in the US (Saikkonen 2000), thus is not adapted to the northern European conditions (Gundel et al. 2013; Saikkonen et al. 2012).

Fertilization decreased reproductive effort and panicle weight, and thus increased the relative panicle number. However, water addition increased panicle weight, and thus reduced relative panicle number, and showed a non-significant tendency to increase reproductive effort. Even though one might expect an increase in reproductive effort as the consequence of higher nutrient availability, alternating peaks of high and low reproductive effort in subsequent growing seasons is likely (Law 1979; Reekie and Bazzaz 2005; Faeth 2009) because reproductive effort also depends on developmental processes along the plants lifetime (Samson and Werk 1986; Weiner 2004; Bonser and Aarssen 2009; Weiner et al. 2009). For example, reproductive allocation may depend on number of vegetative and reproductive meristems and environmental conditions (Saikkonen et al. 1998b; Koivunen et al. 2004; Lehtilä and Sundås Larsson 2005). Seed production in grasses, is progressively generated by vegetative tillers that turn into reproductive phase, and by a variable number of florets (potentially seeds) within panicles (Murphy and Briske 1992). These developmental phases are controlled by environmental cues and specific requirements like photoperiod and vernalization, e.g. shorter days can shorten developmental phases in *Festuca* species (Bean 1970; Murphy and Briske 1992). Furthermore, nutrient addition may promote vegetative growth and tillering while delaying the beginning of the flowering phase (Lehtilä and Sundås Larsson 2005) in a way that developing flowers were exposed to harsh environmental conditions such as freezing temperatures of northern Europe. The consequence of this process which has been termed as “postponement in reproduction” (Weiner et al. 2009), is a lower reproductive effort because there is no time for the plant to allocate and fill all the potentially developed sinks (e.g. panicles, seeds; Gundel et al. 2012). The opposite effect of water on the relative panicle number suggests a different effect of both factors (nutrients and water) on plant development processes.

We have used theoretical background from plant ecology and evolution of plant traits under different ecosystems (Agrawal 2000; Murphy and Briske 1992; Denison et al. 2003) and from plant-microbe coevolution (Ewald 1987; Saikkonen et al. 2004; Thompson 2005) to elaborate our hypothesis and the associated predictions of the effects of fungal endophytes on patterns in biomass allocation and trade-offs in grasses. Even though grass populations commonly exhibit a high frequency of endophyte colonized individuals suggesting a superior relative fitness of endophyte colonized plants over endophyte-free counterparts, the effects of fungal endophytes have been elusive for many plant-endophyte systems (Faeth 2002; Saikkonen et al.

2006; Gundel et al. 2010, 2011). Recent evidence indicates that host plant genotype often overrides the effect of the endophyte in the plant response to different environments (Saikkonen et al. 2010; Cheplick 2011; Vesterlund et al. 2011; Dierking et al. 2012). In addition, the fungal genotype has been found to drive the host plant adaptation to local environments (Hamilton et al. 2010). Those clear effects of endophytes on host plants observed in the past seem to be more complex when considering wild populations that may be more genetically diverse (for the grass and the endophyte), present local adaptation in complex ecological arrangement in the landscape, inefficiencies in the endophyte transmission and migrations among-populations (De Battista et al. 1990; Rice et al. 1990; Hesse et al. 2003; Piano et al. 2005; Saikkonen et al. 2004, 2006; Gundel et al. 2010, 2011; Cheplick 2011; Dierking et al. 2012). Unraveling this complexity will require manipulative experiments that have to be complemented with the genetic information of both the grass and the fungus.

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