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Understanding context-dependency in plant–microbe symbiosis: The influence of abiotic and biotic contexts on host fitness and the rate of symbiont transmission

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ABSTRACT

Understanding the dynamics of a hereditary symbiosis requires testing how ecological factors alter not only the fitness consequences of the symbiosis, but also the rate of symbiont transmission to the next generation. The relative importance of these two mechanisms remains unresolved because studies have not simultaneously examined how the ecological context of the symbiosis influences both costs/benefits and the rate of vertical transmission. Fungal endophytes in grasses have provided particularly tractable systems for investigating the ecological and evolutionary dynamics of hereditary symbiosis. Here we examine interactions between a fungal endophyte, *Epichloë amarillans*, and its grass host, *Agrostis hyemalis*, under altered abiotic and biotic contexts: a gradient of water availability and in the presence versus absence of soil microbes. We show that benefits of the symbiosis were strongest when water was limiting. Symbiotic plants at the lowest watering level produced ~40% more inflorescences and greater seed mass than non-symbiotic plants, while at the highest watering level, symbiotic and non-symbiotic plants did not significantly differ in reproductive fitness. Benefits appear to accrue by allowing hosts to escape from drought, a response that has not been previously reported to be endophyte-mediated. Symbiotic plants at the lowest watering level flowered 9 days earlier than non-symbiotic plants. Interestingly, our results suggest the symbiosis may be costly in the presence of soil microbes, as on live soil, the biomass of symbiotic plants was lower than the biomass of symbiont-free plants. We detected no effect of either the biotic or abiotic context on the rate of symbiont vertical transmission, suggesting that the context-dependent benefits of the symbiosis are the more important driver of variation in symbiont frequency in this system.

1. Introduction

Nearly all plants form symbiotic relationships with microbes (Petrini, 1986; Fitter and Moyersoen, 1996; Saikkonen et al., 1998). These symbionts can alter the ecology of their hosts, often by enhancing nutrient uptake, increasing tolerance to stress, or providing protection from host enemies (Smith and Read, 1997; Clay and Schardl, 2002; Hartley and Gange, 2009). They can also affect community composition (Clay and Holah, 1999; Hartnett and Wilson, 1999), succession (Janos, 1980; Rudgers et al., 2007), and nutrient cycling (Franzluebbers et al., 1999; van der Heijden et al., 2008). Given their potential for strong ecological impacts, not only on host plants but also on the surrounding community and ecosystem, there is great interest in understanding the factors that influence the persistence of these symbioses.

Theory suggests that the persistence of symbionts in host populations is dependent upon the net effect of the symbiont on host fitness and the mode and rate of symbiont transmission (Gundel et al., 2008). Thus, understanding the dynamics of the symbiosis at the population level requires testing how ecological factors alter not only the fitness consequences of the symbiosis, but also the rate of symbiont transmission. Positive, negative and neutral effects of symbionts on plant fitness are expected to arise from variation in the relative costs and benefits of the interaction under different ecological contexts. Variation in the mode and rate of symbiont transmission is also common, with some symbionts transferred horizontally through contagious spread and others transferred vertically from parent to offspring (Bright and Bulgheresi, 2010).

Fungal endophytes in grasses have provided particularly tractable systems for investigating the ecological and evolutionary dynamics of symbiosis. Fungal endophytes occur commonly, can be easily manipulated, and are not obligate for the plant; thus allowing for comparisons of symbiotic and symbiont-free hosts. Within the grass family, Poaceae, approximately 20–30% of species host systemic class 1 endophytic fungi in the fungal family Clavicipitaceae (Leuchtmann, 1992). These symbionts are often vertically transmit-

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ted through seeds of the host plant and can increase host growth and reproduction by enhancing host resistance to both biotic and abiotic stress (Cheplick and Faeth, 2009).

It remains unclear what factors contribute to the context-dependency and long-term persistence of grass–endophyte symbioses. Across host species, there exists substantial variation in the frequency of endophyte symbiosis, both within and among populations (Rudgers et al., 2009), suggesting that endophytes may not be universally beneficial and/or may have low rates of transmission across generations. Experimental evidence generally supports the hypothesis that the outcome of grass endophyte symbioses spans a continuum from parasitism to mutualism, with outcomes dependent upon the biotic and abiotic context (Cheplick et al., 1989; Schardl et al., 2004; Saikkonen et al., 2004; Muller and Krauss, 2005). For example, in tall fescue grass (*Lolium arundinaceum*) the endophyte symbiosis can enhance herbivore resistance (Rudgers and Clay, 2007), competitive ability (Clay et al., 1993) and drought tolerance (Elmi and West, 1995), but can also reduce host biomass under nutrient poor conditions (Cheplick et al., 1989). These context-dependent benefits may ultimately underlie the observed variation in endophyte frequency or persistence. For instance, one study has shown that increased herbivore pressure can drive increases in endophyte frequency (Clay et al., 2005). However, observational evidence suggests that imperfect vertical transmission of symbionts is also common (Afkhami and Rudgers, 2008), and could provide an alternative explanation for variation in symbiont frequency. The relative importance of these two mechanisms remains unresolved because studies have not simultaneously examined both the costs/benefits of the symbiosis and the rates of vertical transmission under altered abiotic or biotic contexts. This is an important step for understanding the degree to which intrinsic dynamics vs. factors extrinsic to the symbiosis influence symbiont frequencies in natural populations.

Historically, endophyte symbioses have primarily been recognized for benefitting host plants through increased resistance to herbivores (Clay, 1996; Bush et al., 1997; Clay and Schardl, 2002). However, research has also shown that endophytic fungi can increase host competitive ability (Clay et al., 1993), drought tolerance (Elmi and West, 1995; Malinowski and Belesky, 2000; Kannadan and Rudgers, 2008), pathogen resistance (Gwinn and Gavin, 1992; Mahmood et al., 1993), and the accumulation of nutrients (Malinowski et al., 2000; Rahman and Saiga, 2005), suggesting these symbionts may play important roles in ameliorating a wide variety of environmental stressors. These alternative pathways of benefits have received less attention than herbivory in the current literature.

Endophyte-mediated benefits to hosts under water stress have been well documented in several agronomically important forage and turf grass species from the genera *Festuca* and *Lolium* (reviewed by Bacon, 1993; Malinowski and Belesky, 2000; Clay and Schardl, 2002; Muller and Krauss, 2005; Saikkonen et al., 2006), and more recently in two native grass species (Morse et al., 2002; Kannadan and Rudgers, 2008). Surveys of native grasses have also documented higher frequencies of symbiosis in drier habitats (Lewis et al., 1997; Leyronas and Raynal, 2001; Novas et al., 2007; Saona et al., 2010), suggesting the potential for a widespread function of endophytes in mediating plant responses to water stress.

Local and seasonal availability of soil moisture is a critical factor determining the distribution and abundance of plant species (Cornwell and Grubb, 2003). Limited water availability can have strong, negative impacts on plant productivity, and plants have evolved adaptations in numerous physiological, developmental, and life history traits to cope with this stress (Geber and Dawson, 1990; Ackerly et al., 2000). These traits have historically been grouped into strategies that enable plants to avoid, tolerate, or escape drought, although it has been recognized that these strategies are not mutually exclusive (Levitt, 1980; Ludlow, 1989).

When subjected to slowly developing water shortages (days to months) some plants can optimize their long-term resource gain through acclimation responses that allow them to avoid or tolerate drought. Avoidance mechanisms allow plants to maintain tissue water potential as high as possible by minimizing water loss or maximizing water uptake. Endophyte-mediated drought avoidance mechanisms have been documented in several species, including changes in the timing and rate of stomatal closure (Elmi and West, 1995; Malinowski et al., 1997), increases in root dry matter (Latch et al., 1985; De Battista et al., 1990; Malinowski et al., 1997), and greater storage of water in tillers (Elbersen and West, 1996; Buck et al., 1997) of symbiotic plants relative to symbiont-free. Endophyte-mediated changes in root traits associated with enhanced water uptake, such as increased root hair length, have also been reported (Malinowski et al., 1999). Tolerance mechanisms allow plant tissues to withstand negative water deficits through changes in physiological and biochemical properties. Endophyte-mediated changes in several drought tolerance mechanisms have also been documented, including the translocation of assimilates to leaves (Richardson et al., 1992), osmotic adjustment (reviewed in Malinowski and Belesky, 2000), and changes in cell wall elasticity (White et al., 1992). As an alternative to acclimation responses, plants can escape drought stress. Escape strategies rely on successful reproduction before the most intense period of drought, allowing plants to maximize fitness. This can be accomplished by increasing growth rates, flowering early, and allocating more resources to reproduction to maximize resource use while water is available. Historically, most attention has been placed on avoidance and tolerance, and, to our knowledge, no investigations have examined the potential for endophyte-mediated escape.

In addition to changes in the abiotic context, the costs and benefits of grass–endophyte interactions may also vary with biotic factors other than herbivory. In this study, we additionally examined the potential role of soil microbes in altering the costs and benefits of the symbiosis. Some prior studies suggested antagonism between foliar endophytes and soil communities, including decreased soil microbial biomass (Jenkins et al., 2006), suppressed plant pathogenic nematodes (Kimmons et al., 1990; Elmi et al., 2000), and reduced mycorrhizal fungi colonization and spore abundance in the soil (Chu-chou et al., 1992; Guo et al., 1992; Mueller, 2003; Omacini et al., 2006; Mack and Rudgers, 2008). Endophyte density has also been negatively correlated with rates of mycorrhizal colonization of roots, and it has been hypothesized that variation in endophyte density could influence rates of vertical transmission of the endophyte (Mack and Rudgers, 2008). While endophytes can clearly have strong impacts on belowground communities and processes, little is known about how microbes belowground influence the costs, benefits, and transmission of these aboveground symbioses.

Here we examine interactions between a fungal endophyte, *Epichloë amarillans*, and its grass host, *Agrostis hyemalis*, under altered abiotic and biotic contexts: a gradient of water availability and in the presence versus absence of soil microbes. Specifically, we address the following questions:

1. Do the costs or benefits of endophyte symbiosis vary with changes in water availability and/or the presence of the soil microbial community?
2. Do these ecological factors influence the rate of vertical transmission of the endophyte?

To our knowledge, this is the first study to evaluate the context-dependency of these two, alternative pathways (costs/benefits, symbiont transmission) that can influence symbiont frequency and persistence.