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Original article

Endophytic benefit for a competitive host is neutralized by increasing ratios of infected plants☆

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ABSTRACT

Leaf endophytes such as *Epichloë* can affect the competitive ability of host grasses, but the reported responses are inconsistent. We hypothesized that this inconsistency is caused, at least in part, by the following two aspects. One is that a competitive advantage might occur as a result of an increase in storage compounds for both growth and defense. Another is that the effect of the endophyte might be related to both water availability and host density. In a greenhouse experiment, we compared the competitive abilities of endophyte-infected (EI) and endophyte-free (EF) *Leymus chinensis*, a dominant grass native to the Inner Mongolia Steppe of China, subjected to ten treatments comprised of a factorial combination of two levels of water availability (well-watered and drought) and five proportions of EI to EF plants (12:0, 4:8, 6:6, 8:4, 0:12). The results showed that the competitive ability of EI plants was higher than that of EF under drought. Here, greater belowground biomass and water use efficiency might contribute to better competitiveness of EI plants. When competing under well-watered conditions, endophyte infection did not provide a benefit to the host plant in biomass accumulation, but more carbon was allocated to defense (total phenolics) in EI plants. This scenario could help EI plants suffer less damage than EF when exposed to herbivores in natural habitats. The competitive ability of EI plants was regulated by EI:EF ratios. Competitive ability of EI plants was higher than that of EF plants in mixtures with lower numbers of EI plants, but the beneficial effect of endophyte infection was neutralized in mixtures with higher numbers of EI plants. Overall, endophyte infection improved the competitive ability of the host under either drought or well-watered conditions but in the presence of herbivore, only this benefit was neutralized by increasing ratios of EI plants. We suspect that both the conditional beneficial effects and stabilizing effects of density differences are likely to keep the endophyte infection rate of *L. chinensis* at an intermediate level.

1. Introduction

Endophytes are microorganisms that colonize internal tissues of plants for all or part of their life cycle without causing signs of tissue damage (Wilson, 1995). Many grasses are systemically infected by clavicipitaceous fungal endophytes in aboveground plant tissues (White, 1987; Clay and Leuchtmann, 1989). Based on numerous studies using tall fescue and perennial ryegrass, this symbiosis has been considered mutualistic – mainly because endophyte infection may improve herbivore resistance of the host grasses due to production of alkaloids (Breen, 1994; Bush et al., 1994; Tanaka et al., 2005; Schardl et al., 2013), increase plant vigor and competitive abilities (Clay and Holah, 1999), and provide increased resistance to a wide range of abiotic environmental conditions such as drought (Elmi and West, 1995; Hesse et al., 2003, 2005; Davitt et al., 2011; Giauque and Hawkes, 2013; Worchel et al., 2013). Increasing evidence suggests that the positive effect of endophyte infection is conditional and can be changed from mutualism to parasitism or commensalism under certain conditions (Saikkonen et al., 2006; Marks and Clay, 2007). Taxonomically, grass endophytes are referred to as *Epichloë* endophytes, among which some are asexual and maternally transmitted by growth into the ovules and seeds of infected plants (formerly referred to *Neotyphodium* (Glenn et al., 1996)), while others reproduce sexually forming cylindrical stromata on the surface of culms, preventing floral development and seed set of the plant (choke disease). Both, asexual *Neotyphodium*

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and sexual *Epichloë* species are now included under the single genus *Epichloë* (Leuchtmann et al., 2014). Leuchtmann et al. (2000) found that sexual *Epichloë* endophytes tended to be free of alkaloids, and those that did produce alkaloids contained only small levels of peramine. In contrast, plants infected with asexual endophytes often contained extremely high levels of lolines. Sexual *Epichloë* endophytes may sometimes reduce host reproductive output, but it is generally unknown if horizontally transmitted endophytes alter the persistence and dominance of the host grass (Brem and Leuchtmann, 2002).

Most grass populations are mixtures of endophyte-infected (EI) and endophyte-free (EF) grasses (Wali et al., 2007; Cheplick and Faeth, 2009). Ecological theory tells us when two closely related species are grown together, their mean yield is determined by both the density and proportion at which the species are growing (Harper, 1977). For closely related EI and EF grasses, individuals experience density-dependent competition with each other as populations grow. The relative strengths of these two classes of competition might determine the potential for their stable coexistence (Chesson, 2000; Miller and Rudgers, 2014). However, reports on how endophyte infection contributes to density-dependent competition are limited (Brem and Leuchtmann, 2002). In the last decade, effects of endophyte infection on competition of the host have been studied intensively in different grass-endophyte systems. A greater competitive ability of plants harboring the endophyte has been documented for tall fescue (Hill et al., 1991), perennial ryegrass (Clay et al., 1993), *Festuca rubra* (Vázquez-de-Aldana et al., 2013) and meadow fescue (*Festuca pratensis*) (Malinowski et al., 1997a; Takai et al., 2010). In contrast, several studies have demonstrated conflicting results that show neutral to negative effects of endophytes on the competitive ability of the host grass (Marks et al., 1991; Richmond et al., 2003; Faeth et al., 2004; Cheplick and Faeth, 2009; Cheplick et al., 2014; Dirihan et al., 2015). All these results suggest that generalizations about the effects of endophytes on host competitive ability cannot yet be made based on the few host species that have been investigated to date (Cheplick and Faeth, 2009; Saikkonen and Helander, 2012). Specifically, there are limited publications that have studied density-dependent competition within and between host types (Brem and Leuchtmann, 2002). When competitive ability is considered, most studies focus only on plant growth characters as response variables (Hill et al., 1991; Brem and Leuchtmann, 2002; Vázquez-de-Aldana et al., 2013). However, the beneficial effect of endophyte infection on the host may not always occur as a change in growth. Endophyte infection may sometimes lead the host to invest more in defense or storage at the cost of reduced shoot growth when resources are limited (West, 1994; Malinowski and Belesky, 2000). Besides alkaloids, many other plant secondary compounds also play important roles in defense (Lambers et al., 1998). Carbon based secondary compounds such as phenolics are general deterrents against herbivores and pathogens (Feeny, 1970), which decrease digestion efficiency of herbivores (Lindroth and Peterson, 1988) and inhibit spore germination of plant pathogens (Grayer and Harborne, 1994). Plants also store nonstructural carbohydrates as energy reserves (i.e. starch and simple sugars) that support recovery from stress and damage (Kobe, 1997). Defense and storage compounds are both carbon-based products of photosynthesis, and plants pay opportunity costs when carbon resources committed to defense and storage are unavailable for growth (Chapin et al., 1990). Different patterns of carbon allocation mean different survival strategies, and optimal allocation successfully reduces the risk of mortality and increases competitiveness (Poorter, 2005). However, up to now, few studies have compared host growth and storage patterns between EI and EF plants (Belesky and Fedders, 1996; Cheplick and Cho, 2003).

The costs and benefits of harboring endophytes not only vary among different endophyte-host species associations, but also change among different environments (Cheplick et al., 2000; Faeth, 2002; Woechel et al., 2013; Oberhofer et al., 2014). Previous studies have shown that asexual *Epichloë* endophyte infection typically improves host growth under water stress both in agronomic grasses (Bacon and White, 1994; Elbersen and West, 1996) and native grasses (Ahlholm et al., 2002; Morse et al., 2002; Kannadan and Rudgers, 2008; Saona et al., 2010; Gibert and Hazard, 2011; Zhang et al., 2011; Oberhofer et al., 2014; Ren et al., 2014; Emery et al., 2015). To expand our understanding of *Epichloë* endophyte infection on competitive ability of native grasses, *Leymus chinensis*, a dominant native grass in the Inner Mongolia Steppe, was chosen as the target of this study. Because high endophyte infection rate only occurs in *L. chinensis* in the Abaga Banner population, where seasonal droughts are normal, we expected that *Epichloë* endophyte may be beneficial by enhancing the competitive ability of infected host plants and thus increasing the proportion of infected plants over time under low water availability. We predicted that advantages might occur in growth, defense or storage. We examined the plant relative growth (biomass), carbon-based defense compounds (total phenolics), and storage compounds (total nonstructural carbohydrates, TNC) of EI and EF plants when they grew together with different proportions under normal and drought treatments. Specifically, we addressed the following questions:

1. Does endophyte infection affect growth, defense and storage of the host grass? And if so, to what extent do they contribute to competitive ability of the host grass?
2. Is the competitive ability of the host grass affected by water availability or EI:EF proportions?

2. Materials and methods

2.1. Study system

Leymus chinensis is a perennial rhizomatous grass. Due to its excellent stress tolerance (Jin et al., 2008), grasslands dominated by *L. chinensis* are widely distributed at the eastern end of the Eurasian steppe. In the middle and north-east parts of the Inner Mongolia steppe, *L. chinensis* is one of the most important dominant species. During a previous investigation, we found that endophyte infection rates in most sites were low, with a range of 0–9.1%. Only in the Abaga Banner population, the west distribution of *L. chinensis* in the Inner Mongolia steppe, was the endophyte infection rate relatively high (63.3%) (Zhu et al., 2013). Endophytes isolated from *L. chinensis* were classified into three morphotypes, and the dominant morphotype was identified as *Epichloë bromicola* (Zhu et al., 2013). In this area, the annual mean precipitation is about 250 mm, which is the lowest among 12 sites of *L. chinensis* populations. *L. chinensis* in this area is dominant species but seldom produce seeds. No stromata have ever been observed in natural populations, thus the endophyte is highly likely transmitted via vegetative propagation. Within this population, plant individuals were sampled with an interval of 5–10 m between them to minimize the probability of sampling ramets belonging to the same genet. The sampling methods have been described in detail in Ren et al. (2014). Thirty EI (infected by *E bromicola*) and 30 EF plants were chosen and maintained in a greenhouse at Nankai University in August 2009.

2.2. Experimental design

For the experiment, we cloned in 600 EI and 600 EF tillers of