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Mycorrhizal Interactions of *Festuca hallii* (Vasey) Piper (Plains Rough Fescue)

by

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are likely to be involved in complex biological interactions as their effects are altered by many biotic and abiotic factors. *Festuca hallii* (Vasey) Piper was used to test whether AMF effects on a single plant species are altered by interactions between neighbour removal or defoliation treatments and local environmental conditions, and whether host life stage affects AMF – neighbour removal interactions. Experiments were performed in, or with soil from, a rough fescue grassland near Kinsella, Alberta, Canada, with AMF suppressed using benomyl. AMF did not alter *Festuca* biomass, and this was not modified by neighbour removal, defoliation, or life stage. However, AMF switched between mutualism and parasitism when certain treatments were applied along specific environmental gradients. That AMF are mutualistic or parasitic on a single host species depending on specific interactions between stressors and local environments suggests that AMF interactions are more nuanced than previously thought.

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GENERAL INTRODUCTION

Natural Complexity and Arbuscular Mycorrhizal Fungi

A fundamental tenet of ecology is that nature is complex. Natural communities are composed of numerous individuals from a variety of taxa, all of which must necessarily interact with their shared environment in order to acquire the various resources required for survival and reproduction. As a result, individuals in natural communities experience multiple, simultaneous interactions with a variety of organisms. Interactions among organisms, particularly plants, are known to be altered by abiotic conditions, adding an additional layer of complexity (e.g., Keddy et al. 1997, Lamb et al. 2007). The combined effects of these various interactions on individuals are often not additive, as the net outcomes integrate multiple simultaneous direct effects, numerous indirect interactions among organisms and abiotic factors, and a variety of feedback loops (e.g., Wootton 1994a, Rosenheim 1998, Lamb 2008). These complex interplays between biotic and abiotic factors, and their combined effects on individuals, are sometimes referred to as multi-way or higher-order interactions (Billick and Case 1994, Wootton 1994b).

Natural complexity, in particular the multi-way interactions described in the preceding paragraph, makes studying the processes structuring natural communities difficult. Statistical packages capable of evaluating the relative importance of, and relationships between, multiple simultaneously-occurring processes and interactions in natural communities have only recently become widely available, and these methods

require very large sample sizes to be effective (e.g., Lamb 2008, Lamb and Cahill 2008). By necessity, then, natural complexity must be broken down and tested in different ways in order to be properly understood. A popular method among plant ecologists for examining processes and interactions of interest is to use pot experiments with only a single or few species and/or individuals (e.g., Keddy et al. 1997, Callaway et al. 2003). The purpose of this type of experimental system is to isolate the interaction(s) of interest by controlling environmental and biotic factors. The strength of this approach also limits its application, however, as it often precludes the sorts of complex, emergent interactions found in nature. As such, one of the main criticisms of pot experiments has been that the results cannot be applied directly to natural systems (Hartnett and Wilson 2002, Gehring and Bennett 2009). In light of this criticism, some researchers favour examining the effects of multi-way interactions within existing communities (e.g., Lamb et al. 2007, Cahill et al. 2008, Casper and Castelli 2007).

One area of research that has recently been the subject of a great deal of interest is the influence of arbuscular mycorrhizal fungi (AMF) on plant population and community dynamics. AMF are obligate plant root symbionts that have classically been viewed as mutualists (Allen et al. 1991). AMF are an ancient lineage, and it has been speculated that the mutualism between plants and AMF may have been partially responsible for allowing plants to successfully colonize land approximately 480 million years ago (see Allen et al. 1991 for an overview). The classical understanding of this symbiosis was that plants provide the fungi with photosynthate in exchange for a variety of benefits, primarily increased phosphorus uptake but also drought resistance and resistance to pathogens, to the ultimate benefit of both organisms (Allen et al. 1991). More recently,

though, it has been demonstrated that the 'mycorrhizal phenotype' – the net effect of AMF on a host plant – exists along a mutualism-parasitism continuum, with the specific position along the continuum determined by a variety of intrinsic and extrinsic factors (Johnson et al. 1997, Jones and Smith 2004). While the basis of the relationship remains the same as was previously understood (i.e., the plant provides carbon to the fungi in exchange for increased nutrient uptake and other benefits), the metabolic costs incurred by mycorrhizal plants may or may not be offset by the benefits conferred by the fungi.

A result of the variable nature of the mycorrhizal phenotype is that AMF are likely to be involved in multi-way interactions affecting their plant hosts. There are a number of factors known to shift the cost-benefit balance of AMF colonization, including soil fertility (e.g., Bethlenfalvay et al. 1983, Hetrick et al. 1990, Bentivenga and Hetrick 1991), fungal and host-plant species-identities (e.g., Hetrick et al. 1990, Klironomos 2003, Klironomos et al. 2004), and other environmental variables (Miller et al. 1995, Subramanian and Charest 1999, Augé 2001, Callaway et al. 2003, 2004). It follows, then, that multi-way interactions involving AMF are likely to occur simultaneously along multiple environmental gradients, and that different species may experience different net effects depending on the composition of the mycorrhizal community. Life stage may also influence an individual's response to AMF colonization. Integration into existing AMF mycelial networks may be especially important for seedlings of some species to establish following germination (Read and Birch 1988, van der Heijden 2004, Stinson et al. 2006). These existing networks are thought to act as 'support systems' by supplying more nutrients to the seedlings than would otherwise be available to them (van der Heijden 2004).

An additional level of complexity is introduced when other organisms besides the host plant and mycorrhizal fungi are considered. For example, the net effect of AMF colonization on an individual undergoing competition is known to be dependent on the species identities of competitors (Grime et al. 1987, Hartnett and Wilson 1999, Scheublin et al. 2007). Plant species with high mycorrhizal dependencies (the percentage gain in biomass when grown with AMF compared with when grown without AMF) relative to competitors generally perform better than species with low mycorrhizal dependencies. The performances of plants with and without AMF therefore depend on plant community composition. Similarly, herbivory may alter the host plant – fungal relationship by lowering photosynthate production. Increased soil nutrient uptake from AMF may allow compensatory growth following defoliation, but reduced photosynthate production increases the relative magnitude of the carbon drain imposed by AMF (Grime et al. 1987, Allsopp 1998). Herbivory may also alter AMF colonization rates (Gehring and Whitham 1994, Eom et al. 2001), with the resulting feedbacks further affecting the cost-benefit balance for host plants (Bever 2002). The mechanisms for this remain unclear as both increased and decreased AMF colonization have been observed, though it is suspected that decreased colonization is a response to decreased photosynthate availability (Gehring and Whitham 1994).

In this thesis, the mycorrhizal interactions of *Festuca hallii* (Vasey) Piper (plains rough fescue) were examined in an effort to understand the degree to which the mycorrhizal phenotype for a single plant species is dependent on interactions among ecological and environmental factors.

Study Site

I conducted my research in, and used soil cores and seed collected from, a native rough fescue grassland at the University of Alberta Kinsella Research Ranch (53°05 N, 111°33 W). This area is part of the Aspen Parkland subregion of Alberta, Canada (Sims and Risser 2000). Rough fescue grasslands are a remnant native plant community characterized by high species richness. Presently, less than 5% of the historic rough fescue grassland range in Alberta remains (Wallis 1987, Natural Regions Committee 2006). The site is a savanna-type habitat consisting of a mixture of rough fescue prairie and trembling aspen (*Populus tremuloides*) forest stands. My work was restricted to the grassland. At the time this research was conducted, the site had never been seeded or tilled, and had not been grazed for two years prior to, nor during, the study. Over 70% of plant biomass was comprised by grasses, while over 70% of plant species diversity was comprised by the forbs (Coupe 2003). Dominant grass species included *Festuca hallii*, *Hesperostipa curtiseta*, *Poa pratensis*, and *Koeleria macrantha*; common forbs included *Achillea millefolium*, *Solidago missouriensis*, *Artemisia frigida*, and *Comandra umbellata* (Shore, *unpublished data)*. Previous research had shown that root competition was strong in this community, with both nitrogen and water availability limiting growth (Lamb et al. 2007). The majority of soils at this site were thin Orthic Black Chernozems, which are grassland soils with thin organic-matter enriched topsoil horizons over glacial till (Howitt 1988, Soil Classification Working Group 1998). The species composition of the AMF community at this site had not been determined.

Common Mycorrhizal Experimental Methods

Mycorrhizal Suppression

The experimental methods used to examine AMF effects on vegetation can for the most part be divided into two categories, which I describe here as 'additive' studies and 'suppressive' studies. In additive studies, mycorrhizal inocula in the form of spores, root cuttings, or infested soil are introduced to sterile soil/growth media. This method is widely employed in pot experiments (e.g., van der Heijden 2004). The benefits of this approach are obvious in that it tightly controls mycorrhizal presence/absence and allows precise manipulation of AMF community composition (e.g., van der Heijden 2004). The major criticism of this methodology, which is often leveled at pot studies in general, is that such a framework is too artificial to be applicable to natural settings (Hartnett and Wilson 2002, Gehring and Bennett 2009). The inclusion of only a single or a few AMF species in mycorrhizal inocula is not representative of the pool of potentially colonizing species in natural systems, and is certainly not representative of the large, shared, pre-existing mycorrhizal mycelial networks that occur in those systems (Read and Birch 1988). Similarly, even if filtrate from unsterilized soil is added back to sterilized soil (e.g., van der Heijden 2004), soil processes are greatly altered from natural systems. A final issue, which may or may not be problematic depending on the context of individual experiments, is that this approach requires that all plants included in the experiments be introduced as seeds or transplants. This precludes the use of established and/or clonal individuals.

In suppressive studies, a systemic fungicide is applied to mycorrhizal vegetation and soils to lower AMF colonization rates (i.e., suppress the mycorrhizal fungi). This

method is often applied in field studies (Harnett and Wilson 2002, see Wilson and Williamson 2008 for an overview), though it has also been used in pot experiments (e.g., Callaway et al. 2003). The primary benefit of employing this method is that it allows AMF studies to be performed in natural systems and/or with naturally occurring AMF communities, thereby incorporating natural complexity and spatial heterogeneity within experiments. There are two major drawbacks to this approach, however, in that fungicide application may cause a variety of non-target effects that may confound interpretation of results, and that AMF are suppressed rather than eliminated in 'non-mycorrhizal' treatments. The extent of AMF suppression may be variable, making interpretation of results difficult.

Benomyl fungicide (methyl 1-[(butylamino)carbonyl]-1H-benzimidazol-2-ylcarbamate) has been the most widely used fungicide for suppressing mycorrhizal fungi in both field and greenhouse studies (see Wilson and Williamson 2008 for an overview). While the cessation of commercial benomyl production by DuPont in 2001 has necessitated finding a suitable alternative, to-date there have been few published studies incorporating other commercially available fungicides (Wilson and Williamson 2008). Benomyl application has been shown to effectively reduce AMF colonization rates and has no direct effects on a variety of plant species in the absence of fungi (e.g., Fitter and Nichols 1988, Hartnett and Wilson 2002; but see Allison et al. 2007). However, while benomyl is effective for reducing AMF colonization, a variety of non-target effects have been reported in long-term addition studies. These include shifts in bacterial- and fungal-feeding nematode densities, bacterial biomass, microbial biomass C, N mineralization, and C:N ratios (Schmidt et al. 2000, Smith et al. 2000). Additionally, non-mycorrhizal

fungi may also be affected by benomyl addition (e.g., West et al. 1993). Since benomyl is a systemic fungicide and is necessarily applied to plant shoots when applied as a soil drench in natural systems, it may reduce both foliar and root pathogenic fungal colonization. This may confound interpretation of results if AMF are thought to be parasitic, as the net effects of AMF cannot be differentiated from those resulting from pathogenic fungi. Proponents of benomyl addition, while acknowledging these concerns, contend that the magnitude of non-target effects is small compared with the effects on AMF colonization (Hartnett and Wilson 1999, 2002, Smith et al. 2000). Additionally, soil sterilization could potentially also cause these or other non-target effects. While the presence of non-target effects is not ideal, there are unfortunately no suitable alternatives to benomyl application for manipulating AMF in natural systems. This topic is discussed further in Chapter 2.

Quantifying Colonization

A general requirement for studies examining mycorrhizal effects on plants is that AMF colonization of roots must be quantified in order to assess the mycorrhizal status of vegetation, and, in the case of suppressive studies, demonstrate the efficacy of the suppression treatment. This is generally done through light microscopy techniques, though biochemical and molecular techniques are becoming more popular (Vierheilig et al. 2005). To perform light microscopy assessment of AMF colonization, the roots segments to be examined are first cleared of pigmentation, acidified with dilute HCl, and then stained with vital or non-vital stains (Vierheilig 2005 for an overview). Though the effectiveness of different stains may vary depending on plant species, the techniques most commonly employed are based on that of Phillips and Hayman (1970), where roots are

cleared using KOH and stained using Trypan Blue (Gange et al. 1999). Epifluorescence has been suggested as an alternative to staining, though the utility of this method has been debated (Vierheilig et al. 2005, Dreyer et al. 2006).

Once roots have been stained, a light microscope is used to score a number of root intersections for the presence or absence of mycorrhizal structures using a standardized technique. Recent studies (e.g., Callaway et al. 2003, Cahill et al. 2008) generally use the magnified intersections method of McGonigle et al. (1990). Prior to development of the magnified intersections method, the grid-line intersect method of Giovannetti and Mosse (1980) had been the most frequently used method for assessing AMF colonization. This method employs a dissecting microscope to examine the mycorrhizal status of root segments where they intersect the lines of a gridded petri dish, but it has been criticized for the ambiguity of some fungal structures at the low level of magnification (40x) used for assessment (McGonigle et al. 1990).

In the magnified intersections method, root segments are mounted in glycerin on a microscope slide, generally with all segments aligned parallel to the long axis of the slide, and covered with a 40 x 22 mm coverslip. The mounted root segments are then observed at 200x using a compound microscope. A number of passes are made perpendicular to the long axis of the slide at regular intervals, and all intact root segments intersected by an eyepiece crosshair are scored for the presence of mycorrhizal structures. The eyepiece crosshair can be rotated perpendicular to the axis of each root segment to give a definitive intersection point. Each intersection is scored for the presence of arbuscules, vesicles, and/or hyphae. Once all passes have been made, the percentage of intersections containing mycorrhizal structures can be calculated. Separate percent colonization rates

can be calculated for the various types of mycorrhizal structures if separate tallies are kept for arbuscules and vesicles. This method is also easily modified to evaluate non-AMF fungal root colonization by calculating the percentage of intersections colonized by melanized hyphae, septate hyphae, and non-mycorrhizal reproductive structures (e.g., Callaway 2003).

Thesis Outline

In this thesis, I applied an AMF suppression treatment in combination with other ecologically relevant treatments to examine the range of variation in mycorrhizal phenotype for a single host species, *Festuca hallii* (plains rough fescue). In Chapter 2, I describe the results of a field experiment at the University of Alberta Kinsella Research Ranch in Kinsella, Alberta, Canada testing whether AMF colonization affected fescue response to neighbour removal and defoliation, and whether the AMF effects were further influenced by natural environmental gradients. In Chapter 3, I describe the results of a growth chamber experiment that used intact soil cores from the Kinsella, Alberta study site to test whether fescue seedlings were differentially affected by AMF colonization in the presence of established competitors as compared with when they were grown alone. Finally, in Chapter 4 I draw conclusions based on the results from Chapters 2 and 3, and suggest directions for future research.

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