# THE ROLE OF MUTUALISTS IN PLANT RESPONSE TO PATHOGEN INFECTION

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Curriculum for the Environment and Ecology.

Chapel Hill

2012

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## ABSTRACT

MEGAN ARLENE RÚA: The Role of Mutualists in Plant Response to Pathogen Infection
(Under the direction of Charles E. Mitchell)

Plants interact with a diversity of microorganisms including enemies and mutualists. Plant pathogens and mutualistic fungi are two classes of microorganisms that directly impact the plant and may in turn alter each other's success. While their roles have often been considered independently, few researchers have considered their concurrent role. Dynamics of these two groups of widespread microbes may modify plant nutrient allocation in response to abiotic environmental changes. Furthermore, early models suggest that mutualists and pathogens may profoundly impact not only their shared host plant, but each other. In one of the first thorough explorations of three-species interactions, I use both experimental and theoretical approaches to investigate the interaction between plants, their pathogenic enemies and fungal mutualists in the context of changing abiotic conditions.

In two separate greenhouse experiments I show that mutualistic strategy is important for determining the direction of change by which mutualists alter pathogen dynamics. In additional work, I also confrim the reverse can also be true in that pathogen infection influences mutualists. Both mathematical theory and an experiment indicate that a pathogen can alter host-mutualist dynamics and consequently alter long-term co-existence of a host and a mutualist. Finally, my thesis shows that changing abiotic

environmental conditions can modify the relationships between hosts, mutualists and pathogens. Using greenhouse and field experiments, I demonstrate that increases in atmospheric $CO_2$, temperature and precipitation all modify mutualist-host-pathogen relationships. Overall, my thesis demonstrates that mutualists and pathogens can have important impacts on not only the host but also on the success of each other. Such dynamics can be further modified by changes in the abiotic environment. Precipitation, temperature, and atmospheric $CO_2$ are all expected to continue to change for the foreseeable future. Thus, in order to make accurate projections about ecosystem, community or population dynamics, changes in microorganisms and their interactions must be included in those projections.

To my family and my friends

whose encouragement and patience have given me the inspiration

and confidence to achieve all of my goals.

## Acknowledgments

The road to my dissertation has been winding with lots of starts, stops, bumps and detours but throughout I have had the support of many people. First, I would like to thank the members of my committee for all of their support, insights and feedback throughout this process. My advisor, Charles Mitchell, has been nothing but supportive in everything I've tried to do. From harebrained experiments to programs in Africa, Charles has never wavered in his commitment to helping me improve as a scientist. James Umbanhowar has always been available for a cup of tea and provided a crucial balance to my Type A personality. I would like to thank Rebecca McCulley and Shuijin Hu for fostering my intellectual development in the mutualists used in this project and hosting me at various points in their lab. I would also like to thank John Bruno and Lauren Buckley for their helpful comments and insights throughout the completion of this dissertation. Finally, I would like to thank Jack Weiss for giving me an ever expanding statistical toolbox.

A variety of organizations and people have supported me throughout my time at UNC. I have had the support of four chairs of the Curriculum for the Environment and Ecology (and its predecessor the Ecology Curriculum) including: Bob Peet, Seth Reice, David Moreau and Jaye Cable. I have also had the support of a number of administrative assistants in the Curriculum including: Cottie Pasternak, Denise Kenney, Mary Beth Robbins and especially Kathleen McNeil. Finally, Valerie Ashby and Kathy Wood with UNC's Alliances for Graduate Education and the Professoriate (AGEP) have always been

there with a well-timed email, free food and general moral support. During my dissertation I have been supported by fellowships from AGEP as well as an NSF Graduate Research Fellowship. I have received additional support via teaching assistantships from the Ecology Curriculum and the Biology Department. AGEP has also provided important travel support throughout my dissertation. Finally, my two writing groups have provided important honest and critical feedback on everything I've written this year. My fellow graduating PhD students Alicia Frame and Josie Reinhardt have helped me navigate this final year with grace, dignity and laughter that I would not have thought possible.

Members of the Mitchell Lab, both past and present, have provided invaluable logistical and moral support throughout this process. Miranda Welsh has been there with me from the start and has been very important to me in many different roles throughout my dissertation. Robert Heckman and Fletcher Halliday have been important in providing support, laughter and motivation when my gas tank was running empty. Our lab technician, Marty Dekkers, has helped me manage all of the different arms of my projects and is always available for a good conversation. Additionally we have been blessed with a stellar crew of undergrads, without whom I would never have been able to get anything accomplished. Gabe Hobsen is a root washer extraordinaire and washed many of the roots for the work presented in Chapter 3. Additionally, Alexandra Chang, Jin Bae, Dan Chen, Lee Dunn, Sarah Hatcher, Drea Paulk, K. Amanda Saunders, Juan Caldron, Bianca White, Angela Ancheta, Carmen Kuan, Tyler Fletcher, Kristine Nguyen, Courtney Whitaker and Xin Wu have all helped with various components of my dissertation. Finally, Briana Whitaker has been the best undergraduate researcher I have ever had the

pleasure of working with. Her hard work and humor never cease to amaze me. She has pushed me to a better mentor and re-invigorated the scientist in me.

Volunteer work has always been important to me, but while at UNC I found it a crucial reminder as to why I became an ecologist in the first place. My fellow volunteers with the Sierra Club Inner City Outings Program have all taught me many things and introduced me to many features of North Carolina during my five years here. Fellow Chapel Hillian Amanda Damweber has always been available for drinks and a bite to eat. Sara Felsen's leadership, laughter and amazing ability to turn camp food into a gourmet meal are nothing short of an inspiration. Rachel Harden-Banyal was the first person to take the time to teach me the flora and fauna of North Carolina. Sarah Johnson and Zach Wilcox are both wonderful with kids and bring imagination back into my life. Last of all, I want to thank the kids who never cease to make me laugh and inspire me with all they've overcome. Together we have conquered the darkness and bugs at Hanging Rock, biked the Raleigh greenway and Tobacco Trail, flipped canoes in Merchant's Millpond, waffed the Eno, learned to swim at Bear Island, Falls Lake and Lake Wheeler, hiked Umstead and attended numerous exhibits and festivals throughout the Triangle.

Fleet Feet Sports in Carrboro has provided an outlet for my energy I didn't even know I needed. Leaving my Southern California roots behind to come to North Carolina not only meant joining the true South (sorry Texas) and initiation into the BBQ wars, but also leaving behind a long history of swimming. Running with Fleet Feet has not only provided a source of exercise but introduced me to a wonderful group of people. Natalie Reder, our fearless leader, is an amazing source of energy at 7am on a Saturday. Katie Cartmell, Anna Lemahieu, Naomi Lundahl, Charlie Bryant and Cara Millonzi have been

there to pick me up when I'm feeling drained and push me that extra mile. Together we completed my first half marathon in March and the Tarheel Ten Miler in April.

Finally, I need to thank my closest friends and family for giving me perspective and keeping me balanced. Vanessa Mendoza has been my best friend since high school JV tennis. Even though she's 5000 miles away, she's always had my back. Hugham Chan has always been a one man cheering squad. Most of all, this dissertation is made possible by the love, support and sacrifices of my family. My mom has always been an inspiration and a strong believer that you're never too old for care packages with gold fish and brownies. My dad's unique humor is a constant reminder to not sweat the small things. Both of my parents have an uncompromising faith in my ability to succeed at whatever I put my mind to and this has helped me through many a tough moment. My brother Colin, like my dad, can always make me laugh and is always there for me when I need him. Even if it means a panicked phone call at 5am Pacific Time (oops). My Aunt Sue has provided the quiet and constant support that only a Minnesotan aunt can provide. During my dissertation I have lost the loving support of my Grandpa whose mind has fully succumbed to dementia and the support of my Abuela who passed from a stroke. It is with their spirit, my grandparents who worked so hard to give their children a different life, my parents who in turn gave me a better life, that this dissertation was completed.

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# CHAPTER I: INTRODUCTION

Plants interact with a diversity of microorganisms, including both enemies and mutualists. Plant pathogens and mutualistic fungi are two classes of microorganisms that directly impact the plant and may in turn alter each other's success. Despite their ubiquitous nature, little research attention has been given to the interaction of multiple microorganisms as they alter host growth and the success of each other. Early three-species models have shown that the third player can alter the intensity, outcome and even the symbiotic state (mutualistic or parasitic) of an association (Bronstein and Barbosa 2002). Furthermore, dynamics of these two groups of widespread microbes may modify plant nutrient allocation in response to abiotic environmental changes. In one of the first thorough explorations of three-species interactions, I used both experimental and theoretical approaches to investigate the interaction between plants, pathogens and mutualists under changing abiotic conditions.

Plant hosts provide an important ecological arena in which to examine multispecies interactions. Specifically, plant phenotypes can be fundamentally altered by microbes, which may provide novel nutritional and defense pathways via their influence on plant biochemical pathways (Friesen et al. 2011). Plant pathogens are one type of enemy which may capitalize on such phenotypic changes (Rúa et al. 2011); however, not all pathogen-plant interactions are created equal. Plants vary in the severity of disease symptoms, perhaps due to differences in tolerance (ability of the plant to recover after

infection), susceptibility (probability of infection when exposed to the pathogen) or resistance (ability of the plant to defend against infection). Often plant tolerance traits are positively associated with traits involved with resource acquisition such as root biomass, the ability to shunt carbon from roots to shoots after foliar damage, leaf area, and photosynthetic rate (Strauss and Agrawal 1999, Stowe et al. 2000). Conversely, tolerance may also be negatively associated with plant resistance traits like concentrations of secondary compounds (Strauss and Agrawal 1999, Stowe et al. 2000).

Variation is common within different enemy-host-mutualist interactions. Few theoretical studies have examined such three-way interactions (but see (Bennett et al. 2006)), and a unifying framework is lacking. Further, most studies consider the plant as the key component for determining the outcome of such three species interactions. Realigning theoretical models to instead utilize the mutualist as the center of focus may prove a more efficient way to explain the large amount of variation surrounding these interactions. Mutualism classes can be grouped based on the nature of the benefits they exchange with their partners (Bronstein and Barbosa 2002): nutritional mutualisms (partners provide each other with essential limiting nutrients) and protection mutualisms (one partner provides protection from biotic or abiotic environmental stresses for the other partner) are two examples of traditionally recognized mutualisms (Bronstein and Barbosa 2002). The fundamental characteristics describing the differences between each of these types of mutualistic relationships may explain some of the variation by which mutualists have been shown to influence plant-enemy interactions.

When exploring the interaction of mutualists and pathogens it is important to study not only the interactions themselves, but their responses under varying abiotic

environments. The extent to which plant-microbe interactions are mutalistic or parasitic may often be a function of resource availability, which is currently being altered by global change. Few studies have directly investigated how such interactions are altered in the context of global change. For example, since viral pathogens and fungal mutualists can be integral players in plant allocation of carbon, the growth, fecundity and population dynamics of these two groups of widespread microbes may modify plant performance in response to elevated $CO_2$ (Malmstrom and Field 1997, Johnson et al. 2005). Additionally, association with one particular class of mutualist - foliar endophytic fungi - may bolster a plant's ability to withstand changes to temperature and precipitation regimes (Stuedemann and Hoveland 1988, Arachevaleta et al. 1989). In order to advance our understanding of host-mutualist-enemy interactions, I investigated the relationship between a viral pathogen and two different types of mutualists (a nutrition mutualist and a protection mutualist) under varying abiotic conditions. I explored multiple aspects of this relationship by combining, greenhouse/lab work, field studies, and a new theoretical model of enemy-mutualist-host interactions. Using greenhouse experiments, we tested the roles mutualists play in altering plant response to viral infection (Chapter 2 and 3). First we considered whether a nutritional mutualist, arbuscular mycorrhizal fungi (AMF), alters host response to pathogen pressure when soil nutrients and atmospheric concentrations of carbon dioxide ($CO_2$) are altered (Chapter 2). We then explored whether a protective mutualist, foliar endophytic fungi, alters host response to pathogen pressure and vector abundance (Chapter 3). The nature of these three-way interactions was further pursued through the use of field studies. We explored the role that endophytic fungi plays in viral disease dynamics under varying precipitation and

temperature regimes within a managed grassland (Chapter 4). Finally, theoretical work exploring multispecies interactions has only recently attempted to dissect enemy-mutualist interactions (Bennett et al. 2006), but previous models have neglected to include pathogens. Since pathogen-mutualist affiliations are characterized by a more intimate connection (primarily they both exist internally in the host), their interactions may exhibit different dynamics then generic plant-enemy relationships. Therefore, we created and analyzed a model of the interaction of a fungal mutualist, a viral pathogen and their shared host in order to explore pathogen-specific influences on three way species interactions (Chapter 5).

**CHAPTER SUMMARIES**

*Chapter 2: Elevated $CO_2$ spurs reciprocal positive effects between a plant virus and an arbuscular mycorrhizal fungus*

While many studies have considered the individual effects of pathogens and mutualists on their hosts, few studies have investigated interactions among microbial mutualists and pathogens in the context of global change. Together with Dr. Kent Burkey at the USDA, Dr. Shuijin Hu at North Carolina State University, Dr. James Umbanhowar at the University of North Carolina at Chapel Hill and my advisor, Dr. Charles Mitchell, I experimentally tested the interactive effects of increased atmospheric $CO_2$ concentration, soil phosphorus supply, an ecologically important nutrition mutualist (AMF), and a widespread viral pathogen. Under elevated $CO_2$, mycorrhizal association increased viral titer, and virus infection reciprocally increased the colonization of roots by mycorrhizal hyphae. This indicates that when carbon was abundant, the mycorrhizal fungus and the virus interacted to stimulate one another's performance. Additionally, virus infection

decreased plant allocation to root biomass, increased the accumulation of phosphorus in leaves, as well as modulated the effects of elevated $CO_2$ and (for one plant species) of phosphorus addition on mycorrhizal colonization of roots. These results emphasize the importance of interactions among multiple microorganisms for plant performance in the context of global change. Overall, our research indicates that these mutualist and pathogenic organisms interact to alter each other's success, and predicts these interactions will respond to changes in resource availability under global change.

*Chapter 3: Fungal endophyte infection and host cultivar jointly modulate host response to an aphid-transmitted viral pathogen*

With Drs. Rebecca McCulley (University of Kentucky) and Charles Mitchell, we investigated how an aphid-transmitted viral pathogen and a protection mutualist (an endophytic fungus) alter host growth and allocation for two different genotypes of the same grass host. While endophyte infection reduced the negative impact of virus infection on root allocation, it also rendered one host genotype more sensitive to the negative impacts of virus infection on tillering. Further, endophyte infection decreased vector production, abundance of adult aphids and total number of aphids on the host, but this did not interact with virus infection status. These results indicate that many of the beneficial effects provided by endophytic infection arise not from the alteration of host interactions with the vectors (aphids), but rather by changing host responses to viral infection. These results highlight the importance of exploring multi-species microorganism interactions at the individual level in order to more fully understand community and ecosystem level interactions.

*Chapter 4: Impacts of climate drivers, host species identity, and fungal endophyte infection on the prevalence of three virus species in a grassland ecosystem*

Under climate change, shifts in precipitation and temperature regimes are expected to impact ecosystem structure and function. These impacts may be determined by feedbacks between plants and their microbes, including both endophytic fungal symbionts and viral pathogens. In collaboration with Drs. McCulley and Mitchell, I considered the role of biotic and abiotic factors in shaping disease dynamics within a managed grassland. After one growing season, all species were tested for infection with three species of barley and cereal yellow dwarf viruses (B/CYDVs). B/CYDVs are aphid-vectored, host-generalist plant viruses that are widespread in natural and agricultural grasslands. Since endophytes produce alkaloids which can deter aphids from feeding, B/CYDV prevalence should be lower in endophyte-infected plants. However, endophyte infection can also confer drought resistance to its host by increasing host water uptake and storage while reducing transpiration loss. This may increase aphid feeding under drought conditions which could increase the prevalence of B/CYDVs despite endophyte induced alkaloids. Thus, how alterations to temperature and precipitation regimes will alter these interactions remains unclear.

Plant species identity influenced risk of pathogen infection, as the odds of infection with one or more B/CYDV viral species were higher for Bluegrass and Dallisgrass compared to Tall Fescue (endophyte-infected or endophyte-free) or Goosegrass. The environmental context provided by abiotic factors also had a strong impact on viral disease dynamics in grasslands. Precipitation decreased overall viral prevalence for plants in this system during the course of the final growing season, but it

had a relative positive effect for endophyte-infected plants. Also in 2011, but regardless of endophyte infection, elevated heat by itself increased virus prevalence for tall fescue. This effect may have been driven by vector population size, as aphid presence was greater in high temperature plots.

Overall, our investigation suggests that disease dynamics in managed grasslands are complex, with both biotic and abiotic factors having important roles. Thus, changes in climate which alter temperature and precipitation regimes are likely to have strong impacts for disease dynamics by not only altering vector presence but also by changing the prevalence of individual viral species; which can scale up to changes in overall disease dynamics.

*Chapter 5: The effect of mutualists on pathogen-host dynamics*

In order to examine the interaction of a fungal mutualist, viral pathogen and their shared host, Dr. Umbanhowar and I created and analyzed a dynamic systems model based on classic Lotka-Volterra model of predation. Both microbes were assumed to alter the uptake and use of soil nutrients by the plant. Qualitative analysis of nullclines demonstrated the presence of threshold dynamics that depend on both the productivity of the system and the strength of the plant-fungal mutualism. In particular, at very low resource availability, plants are obligately dependent on their mutualist to forage for soil resources. Further, we identified complex equilibria states such that the enemy depends on mutualist for persistence, but could also cause the extinction of the mutualist.

In order to more accurately quantify these dynamics, we derived our parameter values from a greenhouse experiment and from the literature, and used them to numerically simulate the system. The plant-enemy dynamics were cyclical, indicating

that the microbes may enhance the abundance of one another or hinder the success of one another. Further parameter exploration demonstrated that if the pathogen is too exploitative it drives the host and fungus extinct. On the other hand, if the fungus is not effective enough as a mutualistic partner, the pathogen can drive the host extinct before the fungus is able to establish. In summation, association with mutualists can alter host-enemy interactions, and the reverse is also true in that enemies may alter host-mutualist interactions.

## CHAPTER II: ELEVATED $CO_2$ SPURS RECIPROCAL POSITIVE EFFECTS BETWEEN A PLANT VIRUS AND AN ARBUSCULAR MYCORRHIZAL FUNGUS


### Abstract

Plants form ubiquitous associations with diverse microbes. These interactions range from parasitism to mutualism, depending partly on resource supplies that are being altered by global change. While many studies have considered the separate effects of pathogens and mutualists on their hosts, few studies have investigated interactions among microbial mutualists and pathogens in the context of global change. Here we experimentally test the interactive effects of increased atmospheric $CO_2$ concentration, soil phosphorus supply, mycorrhizal association and virus infection on the performance of a widespread, ecologically important mutualist and pathogen infecting two wild grass species. Under elevated $CO_2$, mycorrhizal association increased the titer of virus infections, and virus infection reciprocally increased the colonization of roots by mycorrhizal hyphae. Thus, when carbon supply was increased, the mycorrhizal fungus and the virus stimulated one another's performance. These results indicate that plant mutualists and pathogens can alter each other's success, and predict that these interactions will respond to increased resource availability under global change. Additionally, virus infection decreased plant allocation to root biomass, increased the accumulation of phosphorus in leaves, and modulated effects of elevated $CO_2$ and phosphorus addition on mycorrhizal colonization of roots. Overall, this study emphasizes the importance of interactions among multiple microorganisms for plant performance in the context of global change.

## Introduction

Effects of increased atmospheric $CO_2$ on plant growth and productivity are expected to occur both directly via plant physiological responses (Lee et al. 2001) and indirectly via impacts on microbes that associate with plants (Malmstrom and Field 1997, Johnson et al. 2005). Plant pathogens and arbuscular mycorrhizal (AM) fungi are two ubiquitous classes of microorganisms that can directly impact plant allocation of carbon, and may in turn indirectly alter each other's success (Bennett et al. 2006, Smith and Read 2008). Elevated $CO_2$ generally increases the positive impact of AM fungi on plant growth (Treseder 2004). Additionally, elevated $CO_2$ can reduce the negative impacts of pathogen infection on plant growth, increasing disease tolerance (Malmstrom and Field 1997). Together, these studies suggest the potential for interactive effects of plant pathogens and AM fungi on plant performance under elevated $CO_2$. Yet, there have been no studies considering their joint impact on plant performance under elevated $CO_2$. Thus, the goals of this experiment were to explore the independent and interactive effects of a viral plant pathogens, fungal mutualists and changing resource levels as they impact plant performance.

Plants often simultaneously support mutualists and are attacked by natural enemies, creating the potential for interactions that impact plant performance. A meta-analysis of plant-enemy-mutualist interactions concluded that, on average, the presence of mutualists lessens the negative effect of enemies on plant performance (Morris et al. 2007). However, the impact of AM fungi on the performance of plants exposed to natural enemies depended upon the identity of the enemy examined (Borowicz 2001). In addition, the effects of enemy damage on plant performance can depend on the identity of

the plant mutualist (Bennett and Bever 2007). The context dependency and variability highlighted by these studies demonstrates that we cannot safely extrapolate from one type of interaction for another, so understanding interactions between mycorrhizal fungi and plant viruses will require direct study of those systems.

Mutualists and natural enemy interactions are often mediated by their shared host. Theoretical models predict that, by improving plant nutrition and tolerance, AM fungi will also increase enemy populations (Bennett et al. 2006). This prediction may be relevant to effects of AM fungi on plant viruses, with plant phosphorus as the mechanism. Among natural enemies, viruses may be particularly limited by phosphorus availability within hosts because they are comprised chiefly of nucleic acids, which have a relatively high concentration of phosphorus (Clasen and Elser 2007). AM fungi generally increase plant phosphorus concentration under both ambient and elevated $CO_2$ conditions (Smith and Read 2008). Therefore, host plants associating with mycorrhizal fungi may have higher viral titer due to their higher shoot phosphorus content. There is some evidence in agricultural systems which suggest an increase in viral titer as a result of association with mycorrhizae (Daft and Okusanya 1973, Schonbeck 1979), but such reports are limited.

The impact of global change on plant communities may be mediated through indirect effects, including via pathogens (Burdon et al. 2006). Because pathogens do not fix carbon, such indirect effects must begin with effects of elevated $CO_2$ on plant physiology. Although the effect of $CO_2$ can vary considerably across plant species and environmental gradients (Lee et al. 2001), plants grown under elevated $CO_2$ generally show increased levels of photosynthates (Pritchard et al. 1999, Ward et al. 2005),

potentially increasing the resources available to pathogens infecting the host plant (Clasen and Elser 2007, Alexander 2010). Alternatively, elevated $CO_2$ may alter plant-pathogen interactions by changing plant defense traits, including those traits associated with tolerance and resistance (Burdon et al. 2006). Elevated $CO_2$ can alter traits that are associated with pathogen tolerance, the capacity to vegetatively or reproductively compensate for damage by enemies (Strauss and Agrawal 1999). Specifically, elevated $CO_2$ can enhance traits associated with tolerance such as photosynthetic capacity, root biomass, and carbon stores (Strauss and Agrawal 1999, Ainsworth and Long 2005), leading to an increase in plant tolerance of infection (Malmstrom and Field 1997). Overall, changes in plant performance and physiology in response to elevated $CO_2$ may change the growth, fecundity and population dynamics of pathogens (Alexander 2010).

Just as with plant pathogens, alterations of plant physiology due to elevated $CO_2$ may in turn impact mycorrhizal fungi (Johnson et al. 2003, Treseder 2004, Klironomos et al. 2005). The carbon limitation hypothesis suggests that when carbon is limiting, such as can occur under ambient $CO_2$ or under foliar herbivory, AM fungal growth will be reduced because carbon will be preferentially allocated to parts of the plant or soil pool other than AM fungi (Gehring and Whitham 2002). Therefore we expect that elevated $CO_2$ will alter plant physiology to increase the available carbon to AM fungi, thereby strengthening the mutualism by increasing one currency of the mutualism. A meta-analysis of atmospheric $CO_2$ studies found that mycorrhizal fungi consistently and significantly increased their growth in response to elevated $CO_2$ (Treseder 2004). However, mycorrhizae have also been reported to reduce their beneficial effects on plant biomass under elevated $CO_2$ (Johnson et al. 2003, Johnson et al. 2005).

In total, association with mutualists can alter plant-enemy interactions, and enemies can also alter plant-mutualist interactions. In addition, elevated $CO_2$ can alter both plant-pathogen and plant-mutualist interactions. Together, this suggests that elevated $CO_2$ will alter interactions between plant pathogens and mutualists. Yet to date, no experimental studies have examined the effects of elevated $CO_2$ on plants associating with both mutualists and pathogens.

**Materials and Methods**

*Study System*

Barley and cereal yellow dwarf viruses (B/CYDVs) are a group of aphid-transmitted generalist viral pathogens that infect over 150 crop and noncrop grasses (D'Arcy 1995, Halbert and Voegtlin 1995). Infection is systemic and localized to the phloem where it causes necrosis and disruption of carbohydrate translocation (Irwin and Thresh 1990, D'Arcy 1995). BYDV infection stunts plant growth (Malmstrom et al. 2005a), reduces root/shoot ratio (Kolb et al. 1991) and reduces longevity. B/CYDVs are obligately transmitted by aphids, including the globally common aphid species *Rhopalosiphum padi* (L.).

AM fungi are ubiquitous plant symbionts that play an important role in the acquisition of less mobile mineral nutrients, particularly phosphorus (Smith and Read 2008). In return, AM fungi receive carbohydrates from the plant. In addition to altering leaf level photosynthesis (Smith and Read 2008), AM fungi can increase plant root growth (Bryla and Eissenstat 2005).

For this experiment we used two $C_3$ Eurasian annual host plants, *Bromus hordeaceus* and *Avena fatua*, known invaders of Western US grasslands (Malmstrom et

al. 2005b). These host plants were chosen because they are both colonized by AM fungi (Hu et al. 2005, Rillig 2006) and are hosts for B/CYDVs (Malmstrom et al. 2005b). To ensure genetically diverse hosts, experimental seed from multiple wild plants was hand-collected in Oregon and germinated in the experimental pots. When multiple germinates were observed, plants were thinned down to one plant. Plants were watered every three days.

*Experimental Conditions*

The experiment was conducted in the $CO_2$ exposure facility at the USDA-ARS Air-Quality greenhouse at North Carolina State University in Raleigh, NC. The $CO_2$ facility consists of a 9m x 12m greenhouse bay containing 20 continuously stirred tank reactor (CSTR) chambers, each measuring 1.2m in diameter by 1.4 m tall (Chen et al. 2007). Gasses were dispensed and monitored in a laboratory adjacent to the greenhouse. A blower system provided a constant flow of charcoal-filtered air through each CSTR. For those chambers assigned to an elevated $CO_2$ treatment, compressed $CO_2$ was added to the air entering the CSTR. To maintain $CO_2$ at a constant concentration, a rotameter was used to control flow. The potential heating effect of the chambers was alleviated by air which was continuously moved through the CSTR. Monitoring of $CO_2$ concentration was accomplished using computer-activated solenoid valves to direct gas exiting the CSTR into infrared analyzers (model 6252, LiCor Inc., Lincoln, NE, USA).

*Experimental design and treatments*

The experiment was established in a split-plot design with atmospheric $CO_2$ concentration (ambient and elevated $CO_2$) as the whole plot factor with three chambers per $CO_2$ concentration level. Targeted treatments of either ambient or elevated $CO_2$

concentration (ambient + 200 ppm) were randomly assigned to each chamber within a block. The $CO_2$ concentration in elevated chambers is within the range of concentrations predicted by the IPCC for the end of this century (IPCC 2001). Measured values for ambient and elevated $CO_2$ treatments during the study were 387±11 and 581±11 ppm, respectively. AM fungi (mycorrhizal and non-mycorrhizal), virus (infected and uninfected) and phosphorus (addition and ambient) were manipulated as subplot factors at the individual plant level in a full-factorial design.

Individual plants were grown in D60 Deepots (Steuwe and Sons Inc., Oregon, USA). We were interested in the effects of phosphorus and mycorrhizae on plants growing under very nutrient-poor conditions. Each plant received 800 g of steam-sterilized field soil in a mixture of one part sandy loam with two parts of pure sand (by mass). Field soil was collected from a site adjacent to the CSTR facility and steam sterilized to remove any existing soil microbes. The very nutrient-poor soil resulted in slow plant growth, which allowed the plants to grow for an extended period without producing enough biomass to become either light-limited or root-bound. To inoculate plants with AM fungi, we added 50 g of active mycorrhizal spore inoculum per pot. We used commercially available inoculum AM120 from Reforestation Technologies International (Salinas, CA, USA) which consists of the AM fungal species *Glomus intraradices*. Control plants received 50 g of autoclave sterilized inoculum to control for potential changes in nutrient content due to the inoculum. To ensure that, aside from the AM fungus, the same soil microbial community was added to all treatments. All pots received 100 mL of microbial filtrate solution filtrated by Whatman No. 1 filter paper from 10.0 g AM inoculum (in which mycorrhizal spores were removed) to correct for

possible differences in the microbial community and mineral content between mycorrhizal and no mycorrhizal treatments. Plants in the phosphorus addition treatment received 1.42 g of triple super phosphate [$Ca(H_2PO_4)^2$] per pot, mixed into the soil before planting.

To infect plants with virus, we used an isolate of *Barley yellow dwarf virus – PAV* (hereafter referred to as BYDV for brevity) that has previously been used in inoculation experiments (Cronin et al. 2010). This isolate was obtained in August 2007 from a naturally infected *Bromus vulgaris* individual in Oregon; since collection, it has been maintained (approximately three transmission cycles per year) in laboratory plants of the *Avena sativa* cultivar Coast Black Oats. The virus isolate has yet to be sequenced and is not currently in GenBank .Virus inoculations occurred approximately two weeks after germination when plants were at the two leaf stage. Uninfected aphids of the species *R. padi* were fed in petri dishes for 72 hours on infected plant tissue. Five infected aphids were then transferred to each experimental plant, at which time a plastic / nylon mesh cap was placed on plants to prevent the spread of aphids. Aphids were allowed to feed on each experimental plant for 48 hours and then uncapped. Plants were then sprayed with a horticultural oil solution (SAF-T-SIDE, ClawEl Specialty Products, Pleasant Plains, IL) to kill the aphids. Mock-inoculated plants received the same treatment but uninfected aphids were fed on uninfected tissue prior to being transferred to experimental plants. To test the plants for BYDV infection and to quantify relative viral titer concentration, a compound indirect double-antibody sandwich Enzyme-linked Immunosorbent Assay (ELISA; Agdia Inc., Elkhart, IN, USA) was used on 0.1-0.3g wet aboveground tissue collected from experimental plants when they were harvested (Cronin et al. 2010).

Plants were allowed to grow for five and a half months and then harvested. At harvest, plants were separated into above- and belowground portions. Both above- and belowground biomass was placed in a drying oven. Plants were dried at 60˚C for a minimum of 72 hours to obtain dry biomass values. Soils were frozen and stored at -20˚C until they could be processed. The belowground fraction was washed to separate roots from soil. A subset of the roots from each individual were collected before drying, stained with trypan blue following the methods outlined in (Koske and Gemma 1989) and scored for intraradical AM fungal colonization using the magnified gridline intersect method (McGonigle et al. 1990). Using this method, the percentage of root length colonized by intraradical hyphae was measured using a compound microscope (200-400x).

Plant phosphorous concentration was determined using the dry ash/acid extraction method (Stable Isotope/Soil Biology Laboratory of the University of Georgia's Odum School of Ecology).

*Statistical Analysis*

Plants that did not survive or did not become inoculated with the appropriate treatment were eliminated from analyses, resulting in 339 total plants (162 *A. fatua* and 177 *B. hordeaceus).* We used several response variables to assess experimentally induced changes in plant performance. To assess changes in allocation we used root fraction. Root fraction (root biomass divided by total plant biomass) quantifies the portion of the plant's total biomass allocated to roots. We measured root mass and fraction because BYDV is known to have strong negative effects on root biomass of crop plants (Irwin and Thresh 1990, D'Arcy and Burnett 1995) and AM fungi exist within the root portion of the host

plant. Total plant biomass was the sum of all above-and below-ground biomass. To account for the portion of aboveground tissue removed for ELISA, we used a wet/dry conversion factor. To calculate this conversion factor, aboveground material minus ELISA tissue was weighed immediately after harvest and divided by the weight of the same aboveground material after drying. The dry weight for tissue removed from ELISA was then added to complete the total biomass metric. After removing material for ELISA, 91 of the 339 total plants did not have enough plant material for phosphorus analysis and were removed from analyses for this response variable. Thus, analyses considering percent leaf phosphorus as a response variable used 248 plants.

To evaluate specific microbe responses subsets of the dataset were used for analyses. To assess AM fungal response, hyphal colonization can be used as a measure of fungal performance (Smith and Read 2008). Higher percent colonization values can also indicate a greater proportion of plant resource allocation to mycorrhizae (Smith et al. 2009, Smith 2009). To assess treatment-induced changes in hyphal colonization, analyses were limited to only those plants that received active mycorrhizal inoculum and had greater than 5% colonization, resulting in 168 total plants for this response variable. In order to assess viral responses, we used relative viral titer. Viral titer is the concentration of virus present in plant tissue. ELISAs generate optical density (OD) values that can be used as a measure of relative viral titer (Cronin et al. 2010). While compounds in healthy plant sap can influence OD values, comparison of OD values from infected plants with OD values from healthy control plants of the same species indicated that the variation among treatments in OD values from infected plants was several times greater than could be explained by compounds in healthy sap. This indicates that most of the variation in

optical density of infected plants was caused by titer, and therefore our observed optical density data can be used to indicate viral titer. To assess treatment effects on viral titer, analyses were limited to plants indicated infected with BYDV based on ELISA, resulting in 161 total plants for this response variable.

All data were analyzed using R (v.2.13.1, R Foundation for Statistical Computing, Vienna Austria) with the 'lme4' package and the 'lmer' function (Bates and Maechler 2009). Data from the experiment was subjected to analysis of variance using general linear models with appropriate error terms to a split plot design. Response variables were log transformed to fit model assumptions of homogeneity of variances when necessary. Differences within in a treatment were determined using Tukey's HSD with the 'glht' function of the 'multcomp' package (Hothorn et al. 2010). When interactions included plant species, Tukey's tests were performed within each species since main effects already indicated differences between species. Appendix A2 includes full statistical model tables for all response variables.

**Results**

*Viral Titer*

As an indicator of relative viral titer (concentration) in leaf tissue, we analyzed optical density (OD) values from ELISAs. Plant association with mycorrhizal fungi increased the OD of virus infections under elevated $CO_2$, but not under ambient $CO_2$ (AM fungi × $CO_2$ interaction: $F_{1,141}$=4.622, p=0.033; Fig. 2.1). Phosphorus addition decreased OD for *A. fatua*, but not for *B. hordeaceus* (phosphorus × plant species interaction: $F_{1,141}$=4.26, p=0.0409; Appendix A1.1).

*AM Fungal Colonization*

Mirroring the effect of mycorrhizal fungi on relative viral titer, virus infection increased hyphal colonization of roots by mycorrhizal fungi 69% under elevated $CO_2$, but not under ambient $CO_2$ ($CO_2$ × virus interaction: $F_{1,148}$=11.4, p=0.0009; Fig. 2.2A). Looking at the same interaction another way, elevated $CO_2$ increased hyphal colonization of virus-infected plants more than virus-uninfected plants. Further, phosphorus addition decreased hyphal colonization 37% under elevated $CO_2$, but not under ambient $CO_2$ ($CO_2$ × phosphorus interaction: $F_{1,148}$=10.5, p=0.0015; Fig. 2.2B). Phosphorus addition also decreased mycorrhizal colonization of virus-infected *B. hordeaceus*, but not *A. fatua* or virus-uninfected *B. hordeaceus* (virus × phosphorus × plant species interaction: $F_{1,148}$=4.62, p=0.033; Fig. 2.3). Finally, elevated $CO_2$ increased hyphal colonization of both plant species, and more for *A. fatua* than *B. hordeaceus* ($CO_2$ × plant species interaction: $F_{1,148}$=13.6, p=0.0003; Appendix A1.2).

*Plant Biomass and Root Fraction*

Across all treatments and both plant species, virus infection reduced root fraction by 20% ($F_{1,300}$=45.2, p<0.0001; Fig. 2.4A) and tended to decrease total plant biomass by 8.6% ($F_{1,300}$=2.92, p=0.088; Fig. 2.4B). Elevated $CO_2$ increased total plant biomass of non-mycorrhizal *Avena fatua*, but not mycorrhizal *A. fatua* or *Bromus hordeaceus* (AM fungi × $CO_2$ × plant species interaction: $F_{1,300}$=4.4, p=0.037, Appendix A1.3). Across all treatments, *B. hordeaceus* individuals had 52% less total biomass ($F_{1,300}$=79.1, p<0.0001) and 43% smaller root fraction than *A. fatua* ( ($F_{1,300}$=126.2, p<0.0001).

*Leaf Phosphorus Concentration*

Phosphorus addition to the soil increased phosphorus concentration in leaves of both species, and more for *A. fatua* than *B. hordeaceus* (phosphorus × plant species

interaction: $F_{1,257}$=61.9, p<0.0001; Appendix A1.4). Virus infection also increased leaf phosphorus concentration for both species, and more for *A. fatua* than *B. hordeaceus* (virus × plant species interaction: $F_{1,257}$=5.15, p=0.0241; Fig. 2.5). Mycorrhizal fungi did not increase leaf phosphorus concentration ($F_{1,257}$=0.21, p=0.644).

**Discussion**

Changes in abiotic resource supply have been hypothesized to alter plant interactions with microbes (Suding et al. 2008). Our results support this concept, showing that alterations in resource supply can influence performance of both pathogenic and mutualistic plant-associated microbes. In turn, effects on these microbes can influence not only their host but also the performance of each other.

General ecological theory has predicted that host associations with mutualists may increase enemy populations and thus the severity of enemy damage (Bennett et al. 2006). The stoichiometric hypothesis for virus production (Clasen and Elser 2007) leads to a more specific prediction: the association of plants with arbuscular mycorrhizal fungi may increase the titer of virus infections, because AM fungi typically increase host phosphorus concentration (Smith et al. 2009). Our experimental results partially supported this prediction in that virus infections of plants with AM fungi had 20% higher relative titers than did infections of plants without AM fungi (Fig. 2.1). However, AM fungi did not significantly increase host tissue phosphorus (Table A2.5), which suggests that the viral response did not result from the transfer of phosphorous to the plant from AM fungi. This does not completely rule out a role for phosphorus because our phosphorus data was collected at the leaf level, rather than at the level most relevant to the virus, which is restricted to the phloem (Irwin and Thresh 1990, Jensen and D'Arcy

1995). However, physiological mechanisms other than phosphorus transfer may be important. Our finding that AM fungi increased viral titer under elevated $CO_2$, but not under ambient $CO_2$ (Figure 1) suggests that the flow of carbon may also be important in viral production (Malmstrom and Field 1997).

Additionally, the viral pathogen stimulated fungal performance as measured by hyphal colonization (Fig. 2.2A). Specifically, virus infection increased hyphal colonization of roots under elevated $CO_2$. By the same token, elevated $CO_2$ increased hyphal colonization of virus-infected plants more than virus-uninfected plants. Also, virus infection interacted with phosphorus addition to alter fungal performance for one plant species. Phosphorus addition decreased fungal colonization for virus-infected *B. hordeaceus*, but not for virus-free *B. hordeaceus* or for *A. fatua* (Fig. 2.3). Together, these results suggest the possibility that the virus derives a fitness benefit under elevated $CO_2$ by stimulating its host to invest more in a mutualism. While the possible selective pressures behind this are unclear, one possible physiological mechanism involves sucrose conductance via phloem. Typically, B/CYDVs disrupt the flow of carbohydrates, including sucrose flow through the plant (Irwin and Thresh 1990, Jensen and D'Arcy 1995, Malmstrom and Field 1997), which may interfere with or induce the signaling pathways for AM fungi and phosphorous transport. However, in *Avena sativa* grown under elevated $CO_2$, BYDV had the opposite effect on nocturnal reduction of total soluble sugar plus starch in leaves, and in particular virus infection increased the export, respiration, or conversion of sucrose by 30% (Malmstrom and Field 1997). This may have both provided more carbohydrate to AM fungi, and triggered the plant's phosphorus

starvation response, thereby stimulating greater colonization of roots by AM fungi (Smith et al. 2011).

Within host individuals, pathogen populations can be limited by nutrient supplies (Smith et al. 2005, Smith 2007). For example, in an algal-viral system where post-infection viral production was reduced in low-phosphorus host cultures, presumably as a result of insufficient intracellular phosphorus for production of phosphorus-rich viral particles (Clasen and Elser 2007). The universally high phosphorus concentration of nucleic acids, the main component of viruses, suggests that low phosphorus concentration may similarly constrain production and titer of viruses infecting terrestrial plants. This stoichiometric hypothesis predicts that soil phosphorus amendments will increase viral titer in experimental plants. Effects of phosphorus amendment on the prevalence of virus infection in a field experiment were consistent with this hypothesis, although relative virus titer and leaf phosphorus concentration were not analyzed (Borer et al. 2010). In the first experiment to consider the role of BYDV and leaf phosphorus concentration in wild grasses, we demonstrated the reverse in that soil phosphorus amendment significantly decreased relative viral titer for *A. fatua* and had no effect on titer for *B. hordeaceus* (Appendix A1.1). This result indicates that effects of phosphorous supply on viral titer can vary among host species, perhaps depending on their physiological uptake rate or allocation of phosphorus.

In addition to altering microbe performance, changes in resources can also have direct effects on plant biomass and allocation. In a previous study, elevated $CO_2$ increased the biomass of BYDV-infected *Avena sativa* more than uninfected plants (Malmstrom and Field 1997), suggesting that elevated $CO_2$ counterbalances the decrease

in plant carbon uptake caused by BYDV infection. We did not see such a counterbalancing effect in our experiment. This may be because we used two wild host species which have not been selected for agronomic yield, whereas Malmstrom and Field (1997) used *A. sativa*, an agricultural species which could react differently to changes in $CO_2$ availability due to differences in evolutionary history.

Elevated $CO_2$ and mycorrhizal fungal colonization often jointly stimulate plant growth, but such responses can vary with host-fungal species identity (Johnson et al. 2003, Klironomos et al. 2005). In our experiment, elevated $CO_2$ increased total biomass of non-AM-fungal *A. fatua*, but not of AM-fungal *A. fatua*, or of *B. hordeaceus* (Appendix A3). AM fungi had no net impact on total biomass of *B. hordeaceus* or of *A. fatua* plants under elevated $CO_2$, even though elevated $CO_2$ stimulated AM fungal colonization of both plant species (Appendix A1.2). This result suggests that AM fungi did not stimulate plant biomass despite increased activity as measured by hyphal colonization of roots.

Our two study plant species were similar in life history and growth form, as well as in serving as common hosts for mycorrhizal fungi, aphids, and viruses, yet they often differed in their responses to our experimental manipulations. While such differences may be idiosyncratic, study of a larger number of host species may reveal these to be part of a broader pattern. For instance, both *A. fatua* and *B. hordeaceus* fall along a phenotypic continuum in leaf ecophysiological traits which may influence not only the way they respond to biotic factors such as mycorrhizae or pathogen infection, but also to abiotic factors (Wright et al. 2004, Cronin et al. 2010). Further study of the combined effects of abiotic and microbial drivers in such a broader ecological context may be key

to understanding and predicting large-scale changes to ecosystems (Treseder 2004, Suding et al. 2008).

**Acknowledgements**

We are thankful to J. Barton at the CSTR facility for technical help. We would like to thank J. Bever and K. Vogelsang for mycorrhizal inoculum. We are grateful to the Borer-Seabloom lab for collecting and providing all plant seed, and to Jack Weiss for statistical advice. We would also like to thank the Mitchell lab for assistance. This research was partially supported by the joint NSF-NIH Ecology of Infectious Disease program through NSF Grants EF-05-25641 and DEB-10-15909 to C.E.M. and an NSF GRFP to M.A.R.

**Figure 2.1. Effect of mycorrhizal colonization on viral titer.** Across plant species and phosphorus treatment, mycorrhizal colonizaton (+AMF vs. –AMF) increased relative viral titer as measured by Optical Density (OD) value for plants under elevated $CO_2$ but had no effect under ambient $CO_2$. Data shown are means ± SEM; letters indicate significant pairwise differences between means (Tukey's HSD; $p<0.05$).

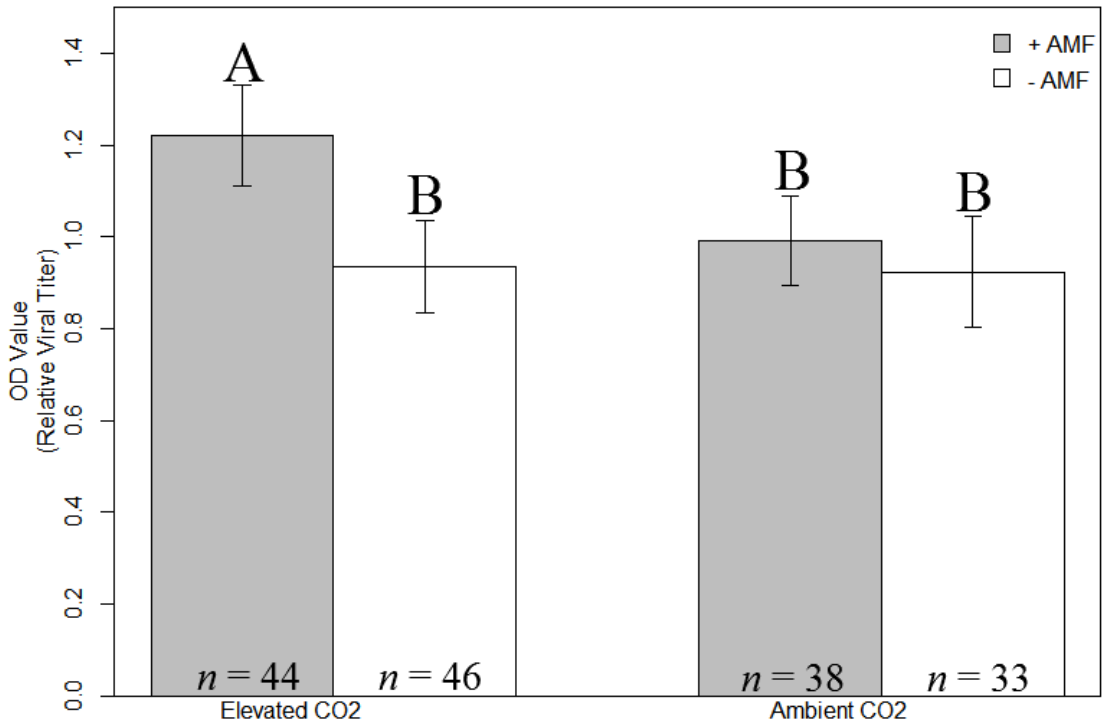


**Figure 2.2. Results for root colonization by AMF.** Across plant species, virus infection increased root colonization by mycorrhizal fungi under elevated $CO_2$ but not under ambient $CO_2$ (A). Phosphorus addition (+P vs. –P) decreased root colonization by mycorrhizal fungi under elevated $CO_2$ but not ambient $CO_2$ (B). Data shown are means ± SEM; letters indicate significant pairwise differences between means within each figure panel (Tukey's HSD; $p<0.05$).

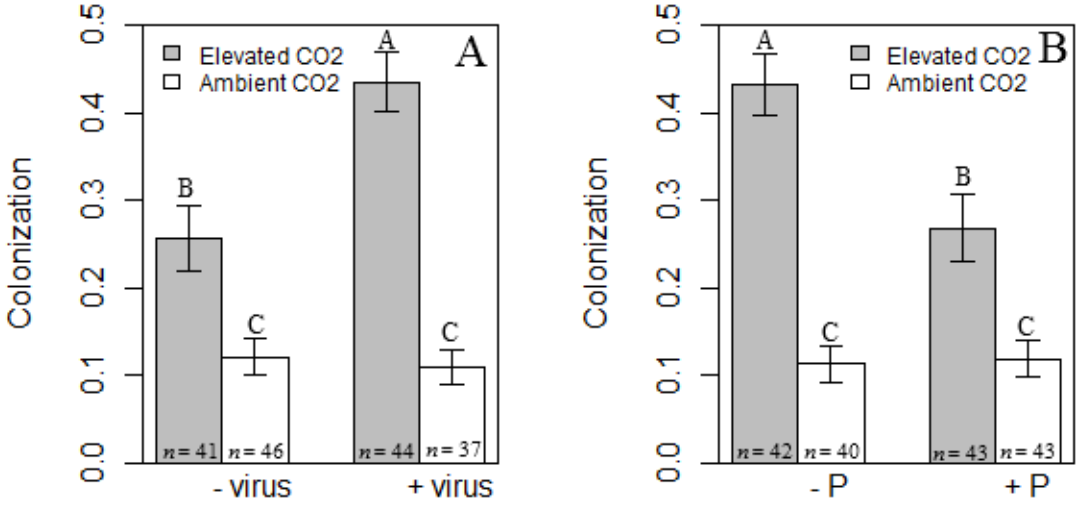


**Figure 2.3. The role of phosphorus, viral infection and host species for root colonization by AMF.** Phosphorus addition (+P vs. –P) did not alter root hyphal colonization for (A) *A. fatua* or (B) virus-uninfected (–virus) *B. hordeaceus* but decreased hyphal colonization for virus-infected (+virus) *B. hordeaceus.* Data shown are means ± SEM; letters indicate significant pairwise differences between means within each figure panel (Tukey's HSD; $p<0.05$).

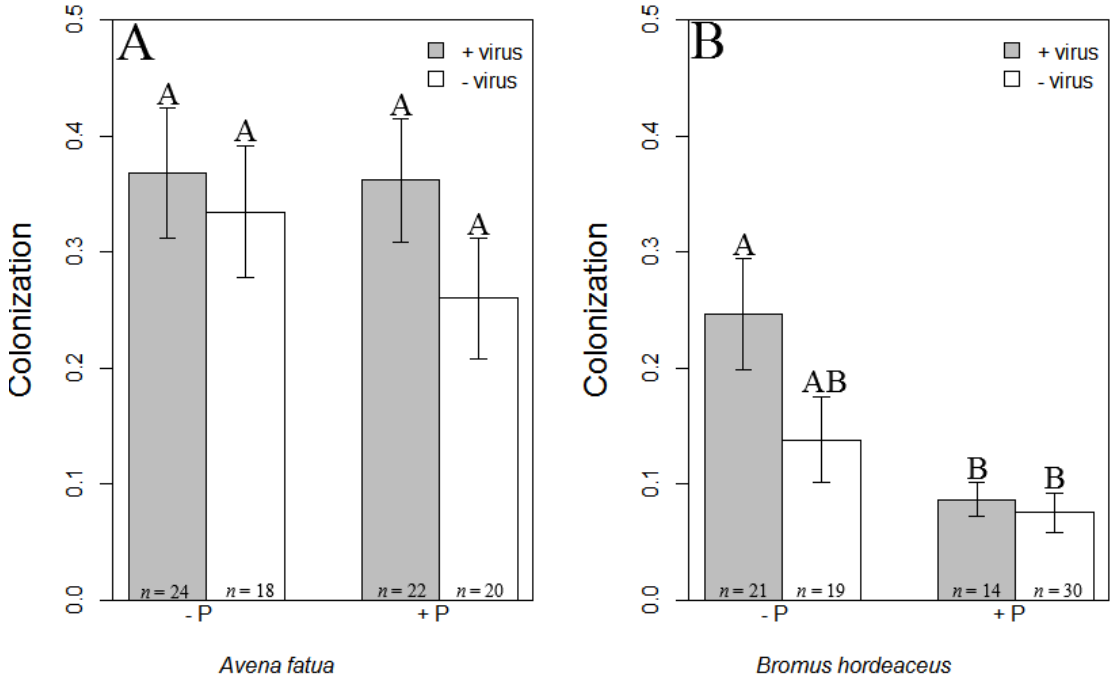


**Figure 2.4. Plant response to virus infection.** Virus infection decreased root fraction (A) and tended to decrease total plant biomass (B). Data shown are means ± SEM.

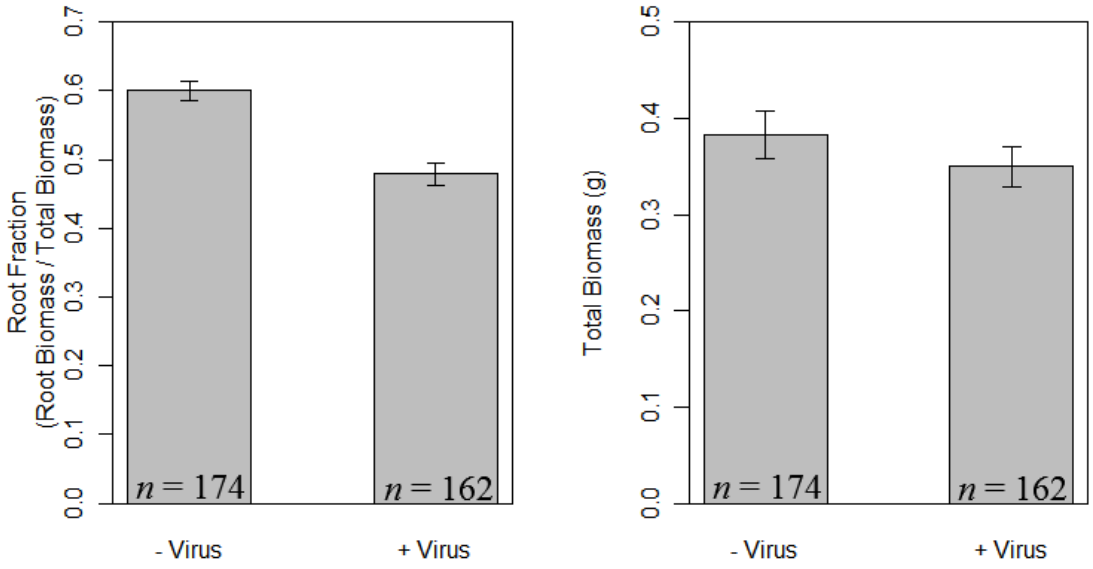
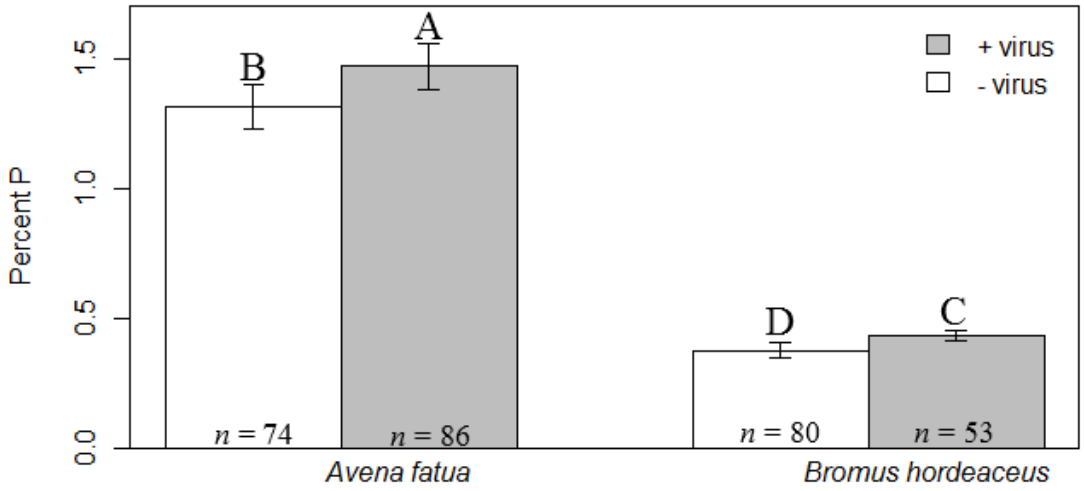


**Figure 2.5. Leaf phosphorus concentration.** Across mycorrhizal status, virus infection increased leaf phosphorus concentration, and more for A. fatua than B. hordeaceus. Data shown are means ± SEM; letters indicate significant pairwise differences between means (Tukey's HSD; $p<0.05$).



## CHAPTER III: FUNGAL ENDOPHYTE INFECTION AND HOST CULTIVAR JOINTLY MODULATE HOST RESPONSE TO AN APHID-TRANSMITTED VIRAL PATHOGEN


### Abstract

1). Despite their ubiquitous nature, interactions between multiple microorganisms and their effect on not only host growth but also one another's success have received limited scientific attention. In this study, we investigated how an aphid-transmitted viral pathogen and a mutualistic endophytic fungus altered host growth and allocation.

2). In a greenhouse experiment, we manipulated endophyte status and virus infection (Barley Yellow Dwarf Virus - PAV) of two tall fescue cultivars. We assessed host, virus and vector responses.

3). Endophyte infection mitigated the negative impact of the virus on root allocation but also allowed the virus to decrease host tillering. Both of these effects had either host or endophyte genotype dependent responses. Endophyte infection universally decreased reproduction and abundance of aphid vectors, and this did not interact with host plant virus infection status.

4). These results indicate that some of the beneficial effects provided by endophyte infection do not arise strictly from altering host interactions with the vector (aphids), but also occur by changing host responses to viral infection. Furthermore, these results emphasize the importance of exploring multi-species microbial interactions and genotype controls on these interactions in order to more fully understand their role in community and ecosystem level dynamics.

## Introduction

Plant hosts are often confronted simultaneously with a diverse array of microorganisms, including both pathogens and mutualists (Arnold 2007, Pieterse and Dicke 2007, Friesen et al. 2011). The close relationships between hosts and their microbes are characterized by a high degree of recognition and signaling between the plant and the associated microbe at molecular, morphological and physiological levels (Harrison 2005). Furthermore, association with microbes can alter plant phenotypes by supplying novel nutritional and defense pathways for the plant as well as influencing plant biochemical pathways (Friesen et al. 2011). Such alterations in plant phenotypes due to association with one microbe may in turn alter plant relationships with other microbes. These relationships may be altered either directly via the shared host or indirectly via a third player such as an arthropod vector. For example, mutualistic microbes can help protect plants against pathogens either by increasing plant defense against pathogens themselves, or by increasing plant defense against herbivores, including arthropods that transmit pathogens (Clay and Schardl 2002, Hartley and Gange 2009). Thus, a broad community context may be important for understanding at least some of these microbial interactions (Saunders et al. 2010). Despite this recognition, few studies examine the impact of interactions among multiple microorganisms on host growth and allocation, or the impact of different microorganisms on each other's success. Here we investigate how the interaction of a foliar endophytic fungus and an arthropod-transmitted plant virus interact with plant cultivar to influence host growth and allocation, and the performance of both the virus and its arthropod vector.

A majority of plant-infecting viruses are dependent upon arthropod vectors for transmission between hosts (Nault 1997, Hogenhout et al. 2008). Therefore, virus ecology is often dependent on the population dynamics, host preference, and movement of vectors (Power and Flecker 2008). Barley and cereal yellow dwarf viruses (B/CYDVs) are a widespread group of aphid-transmitted, generalist viral pathogens that have provided a model system for plant-virus-vector interactions (Gray and Gildow 2003). For example, consumption of B/CYDV-infected host tissue often increases aphid fecundity, with some variation among host, vector and virus species (Power and Gray 1995). Additionally, increased abundance of aphid vectors generally increases the rate at which B/CYDVs are transmitted to healthy plants (Burnett and Gill 1976, Jensen and D'Arcy 1995, Power and Gray 1995). Thus, plant characteristics that alter vector population dynamics are likely to alter their transmission of viruses.

Many agronomic and wild grass species host endophytic fungi in the Ascomycete family Clavicipitaceae. These endophytes receive nutrients, protection, reproduction and dissemination via seeds from the plant (Schardl et al. 2004). In return, the host receives a variety of services from the symbiont including increased soil nutrient uptake (Malinowski et al. 2000) and increased drought resistance (Arachevaleta et al. 1989, Malinowski and Belesky 2000). In addition, many of these endophytes are thought to provide herbivore deterrence via the production *in planta* of several distinct classes of biologically active alkaloids that can reduce arthropod feeding, population size, and consequent damage for the host plant (Clay 1990, Schardl et al. 2004). However, benefits to the host provided by fungal alkaloid production can vary among herbivore species, host species, endophyte genotypes, and host genotype (Cheplick 1998, Faeth 2002).

Endophyte-produced alkaloids may influence aphid-transmitted plant pathogens because, among insect herbivores, aphids are some of the most negatively affected by endophyte infection (Hartley and Gange 2009). Endophytes commonly deter aphid consumption and reduce aphid fecundity (Schardl and Phillips 1997, Hartley and Gange 2009).

For viruses transmitted by aphids and other arthropods, the arthropod deterrence that results from endophyte infection may in turn decrease the severity of virus infection for the host plant. Transmission of B/CYDVs to the plant from the aphid typically requires several hours of aphid feeding (Power and Gray 1995), so decreased aphid feeding duration as a result of endophyte infection may decrease transmission of B/CYDVs to the plant. Furthermore, a decreased number of feeding aphids can decrease the titer of resulting virus infections (Power and Gray 1995), so impacts of endophytes on both aphid population size and feeding duration may reduce the titer of resulting virus infections in endophyte-infected hosts. In turn, reduced virus titer can both decrease the negative impacts of infection on the host plant, and increase the amount of feeding time necessary for uninfected aphids to acquire the virus from the plants (Power and Gray 1995).

Another way in which endophytes may influence B/CYDV infections is through the alteration of biochemical pathways related to pathogen defense. Infection by endophytes may result in mismatches between plant and pathogen signaling, including both toxin-based defenses and recognition-based defenses (Sullivan et al. 2007). For example, initiation of induced defenses against plant pathogens can depend on the recognition of specific pathogen molecules (Voinnet 2005). If endophytes disrupt this

recognition pathway, they broaden the potential for mismatches and result in changes in pathogen protection of the host via endophyte infection.

Within grass-fungal endophyte associations, such as that of tall fescue (*Schedonorus phoenix = Festuca arundinacea*) and *Neotyphodium coenophialum*, endophytes can produce a suite of alkaloid compounds that deters both mammalian and insect herbivory (Schardl et al. 2004). So called 'common toxic' genotypes of these endophytes have been demonstrated to consistently deter arthropods in agroecosystems (Breen 1994), but such deterrence may change with time and abiotic conditions (Hunt and Newman 2005, Rasmussen et al. 2007). 'Novel' forms of some of these endophytes exist and generally lack the ability to produce the mammalian active compounds but retain the compounds important in deterring arthropod herbivores (Malinowski and Belesky 2006). It is possible that host plants infected with novel endophytes may respond differently to virus infection than those infected with the common toxic strain of the endophyte. The limited previous research suggests that novel endophyte infected hosts may be at a competitive disadvantage compared to common toxic endophyte infected individuals when exposed to biotic stresses, such as herbivory, and abiotic stresses, such as variation in growing conditions (Malinowski and Belesky 2006). Additionally, there is evidence to suggest that novel endophytes do not provide the same degree of protection from aphids as common toxic endophytes (Hunt and Newman 2005). Specifically, intrinsic rates of growth for enclosed populations of aphids were greatest for those aphids fed on endophyte-free plants, slower on novel endophyte-infected plants and slowest (or no growth at all) on the plants infected with the common toxic strain of endophyte (Hunt and Newman 2005). Therefore, we predict that novel endophyte

infection will provide less aphid deterrence, and consequently less protection for the host from virus infection, then common toxic endophytes.

Much of the previous research on virus-endophyte-aphid interactions has centered on community-level studies. These studies have generally focused on the impacts of such interactions on agriculturally important host species, with conflicting results. For example, studies that attempted to correlate B/CYDV prevalence and the incidence of endophyte infection for *Lolium perenne* (perennial ryegrass) found no correlation (Guy 1992), while studies considering tall fescue have found that endophyte-infected plants were less likely to be infected by B/CYDVs (Mahmood et al. 1993, Guy and Davis 2002). On the other hand, most plant populations are genetically diverse, and the benefits of endophyte infection can vary among host genotypes (Cheplick 1998). Yet, this previous research has not considered potential impacts of host genotypic differences within the same species. Therefore our research, which examines both host and fungal endophyte genotypic effects of endophyte-host-B/CYDV interactions, can serve to inform both future and past community level explorations of these interactions.

Here, we present the first experiment evaluating the interaction of virus infection and endophyte infection as they relate to impacts on the host. Specifically, we explore how endophyte and host cultivar interact with virus infection to alter vector abundance, host biomass, allocation and tillering. Such impacts are likely to play a crucial role not only in agroecosystems but in natural ecosystems, where fungal endophytes and B/CYDV are also common (Mitchell and Power 2006).

## Materials and Methods

### *Study System*

Barley and cereal yellow dwarf viruses (B/CYDVs) are a group of aphid-transmitted generalist viral pathogens that infect over 150 crop and noncrop grasses (D'Arcy 1995, Halbert and Voegtlin 1995). B/CYDV infection is systemic and localized to the phloem where it causes necrosis and disruption of carbohydrate translocation (Irwin and Thresh 1990, D'Arcy 1995). Impacts of infection include stunted plant growth, reduced root/shoot ratio and reduced longevity (Kolb et al. 1991, Malmstrom et al. 2005a). B/CYDVs are obligately transmitted by aphids, including the globally common aphid species *Rhopalosiphum padi* (L.).

Tall fescue (*Schedonorus phoenix = Lolium arundinaceum = Festuca arundinacea*) is a cool-season grass that has been widely planted for forage in the United States due to its ability to tolerate high temperatures, drought conditions and grazing (Stuedemann and Hoveland 1988). Many of the properties that make S. phoenix attractive for use as a forage species can be attributed to the symbiotic fungal endophyte *Neotyphodium coenophialum* (Clay and Schardl 2002). It is estimated that between 75 and 85% of S. phoenix in the US is infected with the common toxic form of *N. coenophialum* (Ball et al. 1993, Clay and Schardl 2002). Tall fescue provides a valuable model system to investigate microbe-microbe interactions because pair-wise host–fungus interactions and mechanisms for microbe-microbe competition have been well-described in this system (Saunders et al. 2010).

*Experimental design, treatments and conditions*

We used two *S. phoenix* cultivars, KY 31 and PDF. Experimental seed for the KY 31 cultivar was either endophyte free (E-) or contained the common toxic strain of *N. coenophialum* (CTE+). Seed for the PDF cultivar was either endophyte free (E-), infected

with the common toxic strain of endophyte (CTE+), or was infected with a novel strain of *N. coenophialum* (AR 584E+). Seed from the PDF cultivar was obtained from the Noble Foundation in Ardmore, Oklahoma, and seed from the KY 31 cultivar was obtained from the University of Kentucky. Plants were germinated in experimental pots. When multiple germinates were observed, plants were thinned down to one plant per pot. Plants were watered every three days.

The experiment was conducted in the greenhouse at the University of North Carolina at Chapel Hill. In each of the five host-endophyte treatments above, we manipulated virus infection (infected and uninfected) at the individual pot level. This was replicated three times per block for five blocks, yielding a total of 150 experimental plants. Individual plants were grown in D60 Deepots (Steuwe and Sons Inc., Oregon, USA). Each plant received 800 g of steam sterilized soil in a mixture of one part sandy loam soil with two parts of pure sand (by mass).

To infect plants with virus we used the FA2K298 isolate of Barley yellow dwarf virus – PAV (hereafter referred to as BYDV for brevity). This isolate was collected on June 21, 1998 from *Avena sativa* in Central NY State, and has previously been used in inoculation experiments (Power and Mitchell 2004, Hall et al. 2010). Since collection, it has been maintained (approximately three transmission cycles per year) in laboratory plants of *A. sativa* cultivar Coast Black Oats. The virus isolate has been partially sequenced; see GenBank accession numbers DQ285674 and DQ286379 (Hall 2006). Virus inoculations occurred approximately two weeks after plant germination. Uninfected aphids of the species *R. padi* were fed in petri dishes for 72 hours on infected plant tissue. Five infected aphids were then transferred to each experimental plant, at which time a

cap, constructed of clear plastic and nylon mesh, was placed on plants to prevent the spread of aphids. Aphids were allowed to feed on each experimental plant for 48 hours and then uncapped. In order to assess vector feeding responses, the number of apterous (unwinged) adult aphids, alate (winged) adult aphids, and juvenile nymph aphids (whether apterous or alate) were counted for each plant. Plants were then sprayed with a horticultural oil solution (SAF-T-SIDE, ClawEl Specialty Products, Pleasant Plains, IL) to kill remaining aphids. Mock-inoculated plants received the same treatment, but uninfected aphids were fed on uninfected tissue prior to being transferred to experimental plants. To test the plants for BYDV infection and to quantify relative viral titer (concentration), a compound indirect double-antibody sandwich Enzyme-linked Immunosorbent Assay (ELISA; Agdia Inc., Elkhart, IN, USA) was used on 0.1-0.3 g wet aboveground tissue from experimental plants (Cronin et al. 2010). Five plants that were inoculated with infected aphids but did not become infected with BYDV were removed from the analysis for a total of 145 experimental plants.

Plants were allowed to grow for six weeks after inoculation and then harvested. At harvest, plants were separated into above- and below-ground portions. Soils were frozen and stored at -20˚C until they could be washed. The belowground fraction was washed to separate roots from soil. Both above- and below-ground biomass samples were oven-dried at 60˚C for a minimum of 72 hours to obtain dry biomass values.

*Statistical Analysis*

We used several response variables to assess experimentally induced changes in plant performance. To assess changes in plant allocation, we used root fraction, root biomass divided by total plant biomass. BYDV is known suppress root allocation (Irwin

and Thresh 1990, D'Arcy and Burnett 1995); therefore, this root fraction is an important indicator of virus impact. Total plant biomass was the sum of all above- and below-ground biomass. To account for the portion of above-ground tissue removed for ELISA, a wet/dry conversion factor was calculated based on the ratio of wet/dry biomass and applied to the ELISA weight. This estimated dry mass was then added to complete the total biomass metric. We quantified tillering, a component of vegetative growth that can be sensitive to damage from natural enemies (Jewiss 1972), by counting the number of tillers per plant. In order to assess viral responses, we used relative viral titer. Viral titer is the measure of the concentration of virus present in plant tissue. ELISAs generate optical density values that can be used as a measure of the relative viral titer (Cronin et al. 2010). To assess the impacts of endophyte genotype and host cultivar on viral titer we considered only those plants infected with virus.

We performed two sets of statistical analyses to answer two different sets of questions. In order to assess cultivar x endophyte interactions, we excluded plants of the PDF cultivar infected with the novel endophyte AR 584 because there was no equivalent cultivar-endophyte combination for the KY 31 cultivar. For the same reason, to assess the role of endophyte cultivar in altering plant-virus-vector interactions, we excluded plants of the KY 31 cultivar and considered only the PDF cultivar.

All data were analyzed using R (v.2.13.1, R Foundation for Statistical Computing, Vienna Austria) with the 'lme4' package and the 'glmer' and 'lmer' functions (Bates and Maechler 2009). Data from the experiment was subjected to analysis of variance using general linear models with greenhouse block as a random effect. Response variables were log transformed to fit model assumptions of homogeneity of variances when necessary.

Differences within a treatment were determined using Tukey's HSD with the 'glht' function of the 'multcomp' package (Hothorn et al. 2010). Appendix B2 includes tables of the full statistical models for all response variables.

**Results**

*Plant Biomass and Allocation*

Common toxic endophyte infection significantly decreased total plant biomass of the KY 31 cultivar, but tended to increase total plant biomass of the PDF cultivar (endophyte x cultivar: $F_{1,105}$ =5.794, p= 0.018, Fig. 3.1A). Within the PDF cultivar, total plant biomass did not differ between plants infected with novel vs. the common toxic endophyte genotypes (Tukey HSD: p=0.938); however, endophyte-free plants produced less biomass than plants infected with the common toxic endophyte (Tukey HSD: p=0.043; Fig. 3.1B). Across all endophyte and host cultivar treatments, virus infection decreased total plant biomass by 70% ($F_{1, 105}$= 15.65, p = 0.0001). Virus infection decreased both root ($F_{1,105}$=21.1, p <.0001) and shoot biomass ($F_{1,105}$=11.94, p= 0.0008), neither of which was significantly altered by common toxic endophyte infection alone (p > 0.7). However, while virus infection decreased the root fraction of endophyte-free plants (Tukey HSD: p=0.0097), common toxic endophyte infection greatly reduced the magnitude of this effect and rendered it statistically non-significant (Tukey HSD: p=0.3648 Fig. 3.2). Within the PDF cultivar, the fungal endophyte genotype had no significant effect on root fraction ($F_{2,79}$ = 0.2902, p = 0.749) or interaction with the virus ($F_{2,79}$ = 2.098, p = 0.1295).

*Tiller Number*

Virus infection decreased the number of tillers produced per plant for common toxic endophyte-infected plants from the KY 31 cultivar, but not endophyte-free KY 31, or the PDF cultivar regardless of endophyte status (virus x cultivar x endophyte: $z = 2.329$, $p = 0.0198$; Fig. 3.3A). Across virus treatments, infection of PDF by either the common toxic ($z = 3.663$, $p = 0.0003$) or the novel endophyte ($z= 1.900$, $p = 0.0575$) increased the number of tillers produced compared to the endophyte-free PDF plants, but there was no difference in tiller number between plants infected by the novel endophyte or the common toxic endophyte ($z=-0.880$, $p = 0.6530$, Fig. 3.3B).

*Aphid Abundance*

Across host cultivars, common toxic endophyte infection decreased the number of nymphs ($z = -1.882$, $p = 0.059$; Fig. 3.4A), apterous adult aphids ($z = -2.162$, $p = 0.031$; Fig. 3.4B) and the number of total aphids (nymphs + apterous adults + alate adults; $z = -3.068$, $p = 0.0022$; Fig. 3.4C). Production of aphid nymphs (juveniles) was lower for those aphids that fed on the PDF cultivar ($z = -2.557$, $p = 0.0106$) compared to those that fed on KY 31, and for those aphids that fed on virus-infected vs. virus-free tissue ($z = -2.965$, $p = 0.003$). There were no treatment interactions that significantly influenced nymph abundance ($p > 0.2$). Within the PDF cultivar, endophyte infection and genotype did not influence aphid nymph abundance ($p >0.9$), aphid adult abundance ($p > 0.7$) or total aphid abundance ($p > 0.7$).

*Viral Titer*

As an indicator of relative viral titer in leaf tissue, we analyzed optical density (OD) values from ELISAs for virus-infected hosts only. Averaged across endophyte statuses, optical density values for the KY 31 cultivar were 91 percent higher than for the

PDF cultivar ($F_{1,48}$=11.98, p = 0.0011; Fig 3.5). On average, OD values were 33% lower in endophyte-infected plants than in endophyte-free plants, but this was not statistically significant ($F_{1,48}$=1.775,p = 0.1891), and there was no effect of endophyte infection on ODs within either the KY 31 cultivar ($F_{1,23}$ = 1.549, p = 0.2259) or within the PDF cultivar (p > 0.30).

**Discussion**

While BYDV infection universally reduced plant biomass in our experiment, our results indicate that endophyte infection benefited the plant by reducing the severity of virus impacts on belowground plant allocation. Further, endophyte infection had impacts on vector abundances, although such impacts did not translate to significant impacts on viral titer. Finally, virus infection, endophyte infection, and host cultivar interacted to control production of new tillers, a key component of growth in perennial grasses such as tall fescue.

Since arthropod vectors play a pivotal role in the transmission of most plant viruses (Power and Flecker 2008), we predicted that the arthropod deterrence that results from endophyte infection (Schardl et al. 2004) would decrease the severity of virus infection for endophyte-infected hosts. Specifically, we predicted that endophyte presence would lower virus titer by decreasing aphid feeding time and production (Power and Gray 1995). Lower titers should then result in plants that are less severely impacted by virus infection than endophyte-free, viral-infected plants. In terms of total plant biomass, our results did not support this hypothesis, as total biomass was not influenced by endophyte-virus interactions. But in terms of biomass allocation, our results were consistent with the hypothesis because endophyte infection greatly reduced virus impacts

on root allocation. While infection with B/CYDVs typically decreases root allocation for infected hosts (Irwin and Thresh 1990), common toxic endophyte infection ameliorated this effect. This change in host growth may allow common toxic endophyte-infected hosts to survive longer and tolerate virus infection better in a field setting.

Our results indicate that host cultivar is also important for considering virus-endophyte interactions. Relative viral titer was much higher in the KY 31 cultivar than in the PDF cultivar. While endophyte infection tended to increase overall plant biomass for plants from the PDF cultivar, endophyte infection significantly decreased overall plant biomass for the more common KY 31. Furthermore, the relationship between endophyte infection and host cultivar in response to viral infection was important for determining the number of tillers produced. While virus infection decreased the number of tillers produced for endophyte-infected plants in the KY 31 cultivar, there was no corresponding effect of virus infection for endophyte-infected plants in the PDF cultivar. This result may reflect variation in alkaloid and metabolic profiles among different host genotype-endophyte combinations within the same host species (Faeth et al. 2002, Rasmussen et al. 2008). Differences in alkaloid profiles may explain why endophyte infection can increase herbivory on some host species (Faeth and Shochat 2010, Jani et al. 2010) while typically decreasing herbivory in other host species (Clay and Schardl 2002, Schardl et al. 2004, Saikkonen et al. 2010). Furthermore, differences in metabolic profiles due to host genotype-endophyte interactions are substantial, contributing to differences in herbivory (Rasmussen et al. 2008), maybe even more so than alkaloid production (Rasmussen et al. 2009). Thus, it is possible that the alkaloid and metabolic

profiles of different host-genotype-endophyte combinations can have differential effects on the virus and/or the vector.

The primary mechanism by which we predicted virus-endophyte interactions to occur is via alterations to the arthropod vector. Consumption of B/CYDV-infected host tissue commonly increases aphid fecundity (Jensen and D'Arcy 1995), but endophytes deter aphid consumption and fecundity (Schardl and Phillips 1997, Hartley and Gange 2009). As expected, endophyte infected plants, both common toxic and novel genotypes, supported lesser aphid production, abundance of adult aphids and total number of aphids. There were also additional differences due to host cultivar in which the PDF cultivar decreased production of aphid nymphs compared to KY 31. Contrary to some previous work (Jensen and D'Arcy 1995), virus-infected plants produced fewer nymphs than virus-free plants. Phenology of infection (particularly the degree of phloem degeneration) has been identified as an important driver of aphid response to plant infection (Gildow 1983, Power and Gray 1995). Therefore, changes in the phenology of infection due to either cultivar or endophyte association may be responsible for decreasing aphid abundances on virus-infected plants.

A typical characteristic of the host-endophyte association is the production of herbivore-deterring alkaloids, which can have detrimental effects on mammals with the common toxic form of the endophyte (Schardl et al. 2004). It has previously been shown that novel endophytes can invoke different degrees of protection from herbivores and environmental stresses compared to the common toxic strain (Hunt and Newman 2005, Malinowski and Belesky 2006). In our study, novel endophytes and common toxic endophytes did not invoke different host responses to viral infection or differences in

aphid reproduction. Within the PDF cultivar, we saw no differences in nymph, adult or total aphid abundance due to the presence of the novel endophyte compared to the common toxic endophyte infected and endophyte-free plants. Additionally, the common and novel endophytes increased overall biomass and tiller production similarly compared to endophyte free plants. . This indicates that in our study, the novel endophyte AR 584 provided the same degree of benefit in terms of pathogen protection from B/CYDVs as the common toxic endophyte for this host cultivar. Furthermore, there were no significant interactions between virus infection and endophyte genotype for overall plant biomass, root fraction or tiller production. Thus, the different alkaloid profiles produced by these two endophytes do not appear to be important in altering virus interactions, suggesting that alkaloids are not the mechanism for these interactions.

In conclusion, these results indicate that much of the benefit endophyte infection provides plants exposed to insect-transmitted virus infection arises not from decreasing vector fecundity and abundance, but rather from altered host biomass allocation in response to virus infection. Thus our work provides a largely unconsidered, but perhaps general, mechanism by which one microbe can alter plant phenotypic response to other microbes, and also illustrates the complex genotype interactions between the plant host and fungal endophyte that challenges our understanding of this system (Friesen et al. 2011).

**Acknowledgements**

We thank the Noble Foundation for providing the PDF material and Dr. Tim Phillips at the University of Kentucky for the KY 31. We are grateful to Jack Weiss for statistical advice. We would also like to thank the Mitchell lab for assistance. Lauren

Buckley, Alicia Frame and Josie Reinhart provided comments on earlier versions of this manuscript. This research was partially supported by the joint NSF-NIH Ecology of Infectious Disease program through NSF Grants EF-05-25641 and DEB-10-15909 to C.E.M., Kentucky Agricultural Experiment Station funds (KY006045) to R.L.M. and an NSF GRFP to M.A.R.

**Figure 3.1. Biomass response to endophyte infection.** Common toxic endophyte infection decreased total plant biomass for the KY 31 cultivar but did not have a significant effect on the PDF cultivar (A). Within the PDF cultivar, infection with the novel endophyte increased total biomass in comparison to endophyte-free plants (B). Data shown are means ± SEM; letters indicate significant pairwise differences between means within each panel (Tukey's HSD; $p<0.05$).

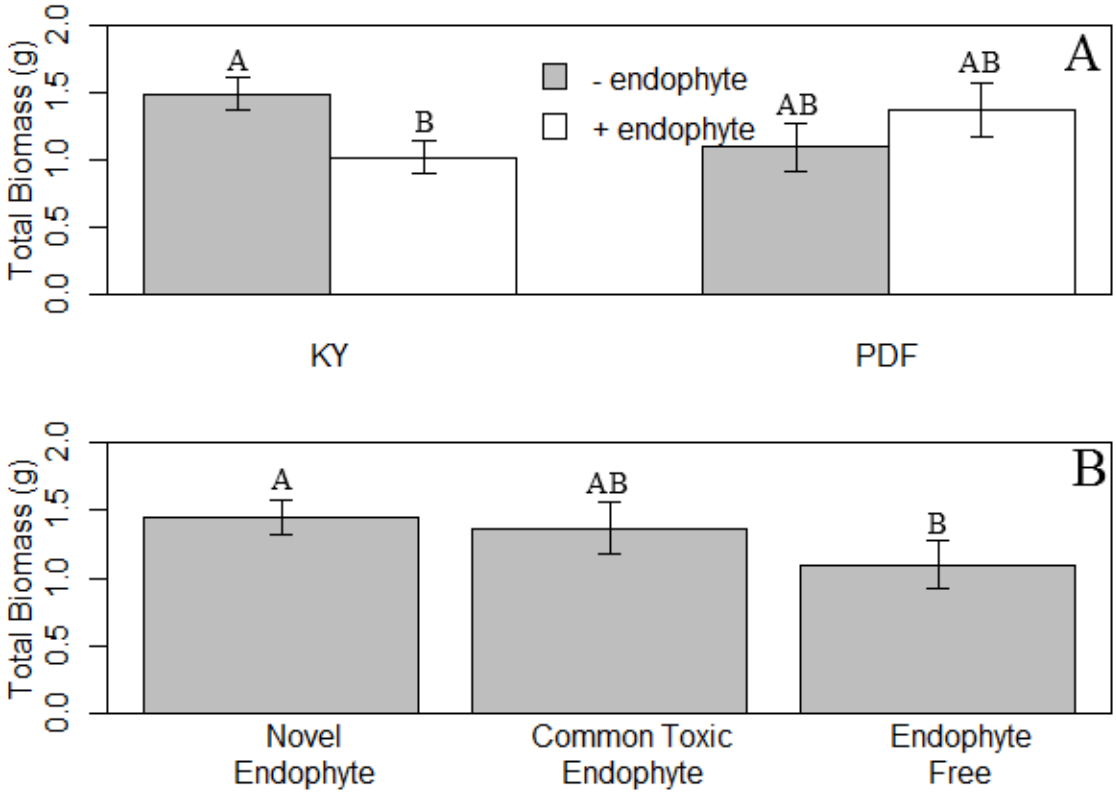


**Figure 3.2. Root Fraction by virus and endophyte infection**. Across host cultivars and considering only the common toxic endophyte genotype, virus infection decreased root fraction of endophyte-free plants but not of endophyte-infected plants. Data shown are means ± SEM; letters indicate significant pairwise differences between means (Tukey's HSD; $p<0.05$).

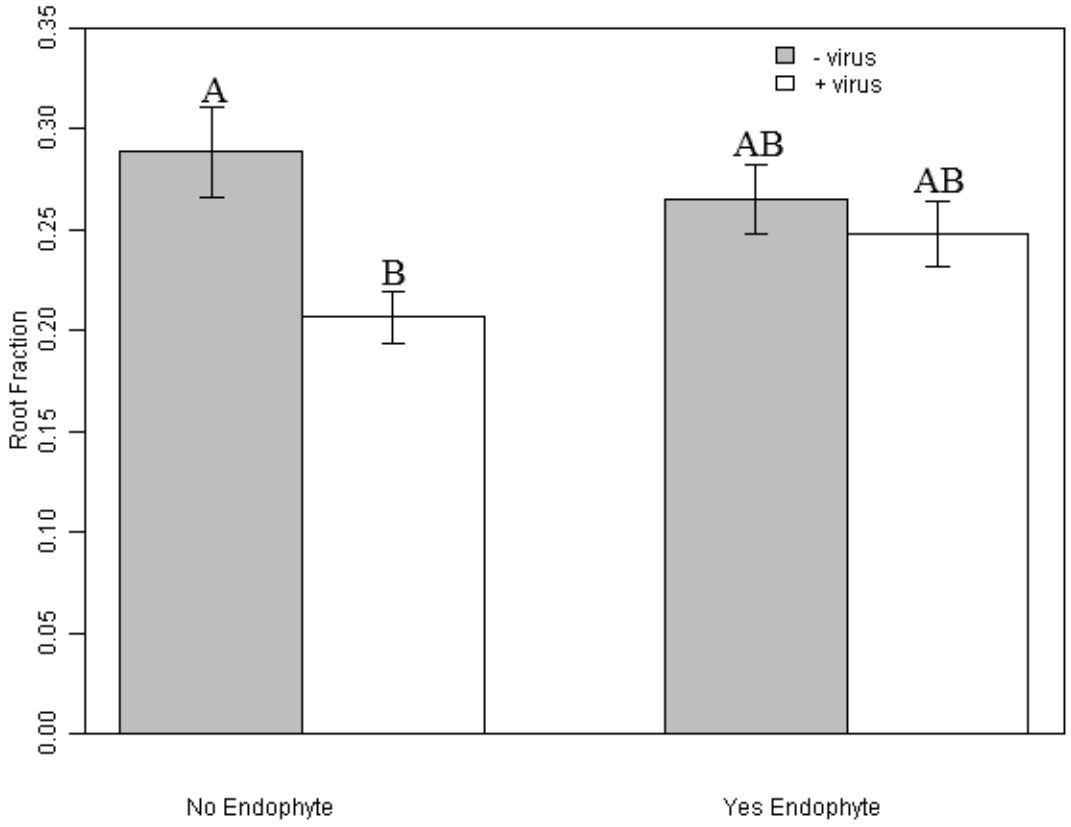


**Figure 3.3. Average tiller number by endophyte and virus infection status.** Considering only the common toxic endophyte, virus infection significantly decreased tiller production for endophyte-infected plants in the KY 31 cultivar only (A). Across virus infection status, infection with the novel endophyte or the common toxic endophyte increased tiller production for the PDF cultivar (B). Data shown are means ± SEM; letters indicate significant pairwise differences between means (Tukey's HSD; $p<0.05$).

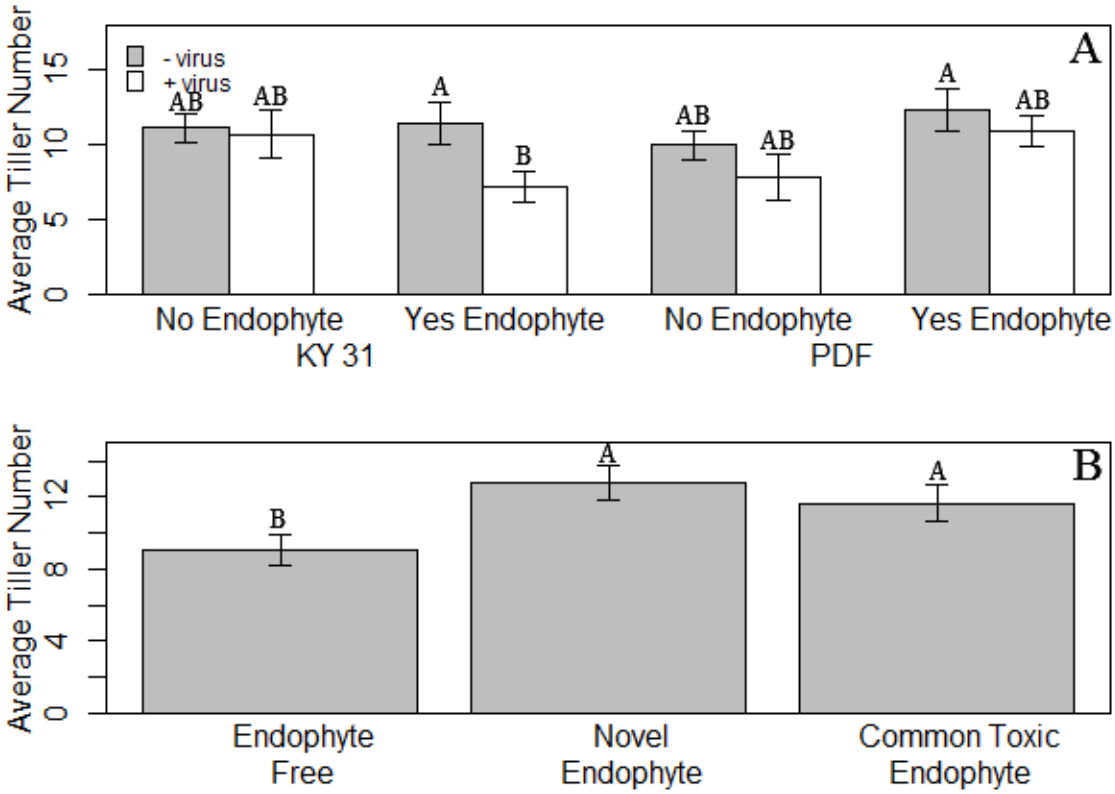


**Figure 3.4. Viral titer by genotype.** Averaged across host cultivar, common toxic endophyte infection ('y') decreased the abundance of aphid nymphs (A), abundance of apterous adult aphids (B), and total abundance of aphids (C). Data shown are means ± SEM.

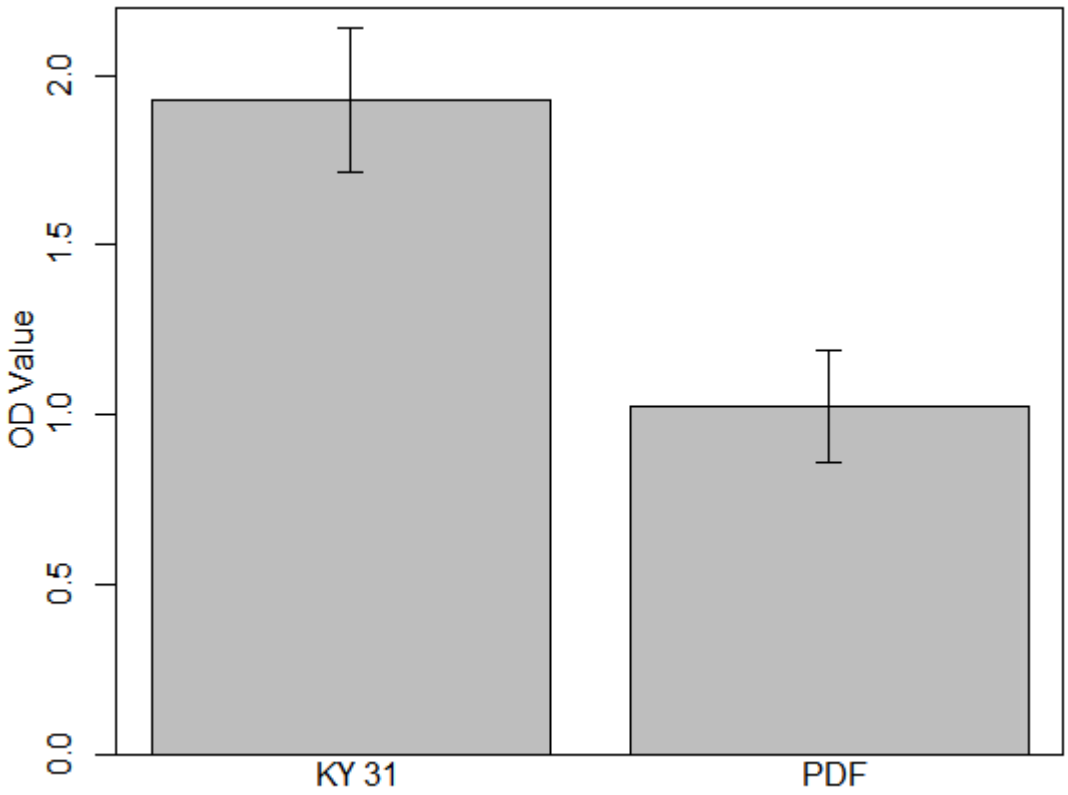
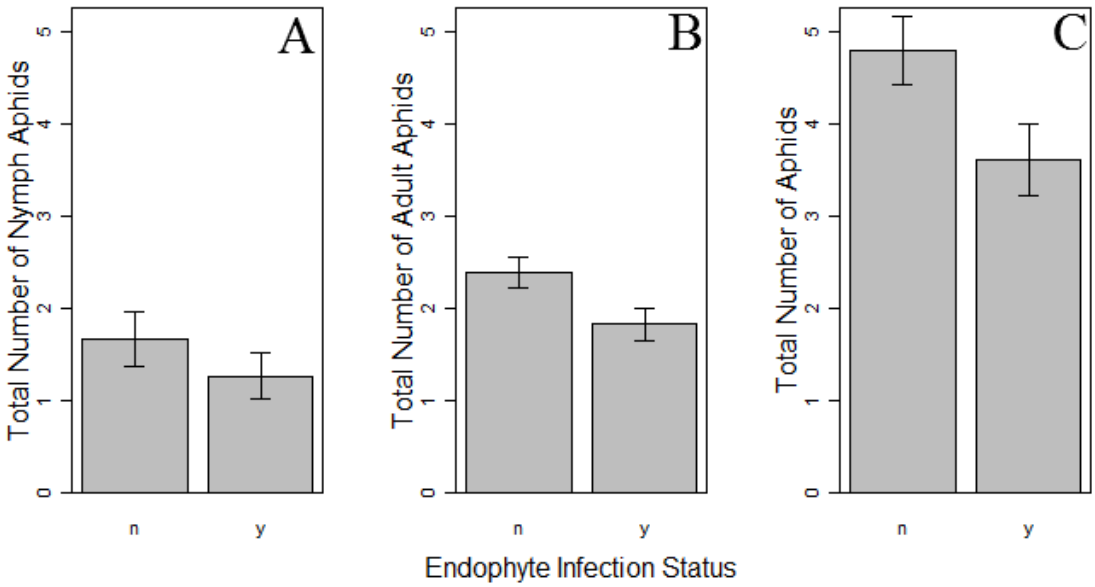


**Figure 3.5. Aphid response to endophyte infection.** The KY 31 cultivar had significantly higher relative viral titer as measured by Optical Density (OD) value then the PDF cultivar. Data shown are means ± SEM.



# CHAPTER IV: IMPACTS OF CLIMATE DRIVERS, HOST SPECIES IDENTITY, AND FUNGAL ENDOPHYTE INFECTION ON THE PREVALENCE OF THREE VIRUS SPECIES IN A GRASSLAND ECOSYSTEM


## Abstract

Under climate change, alterations to precipitation and temperature regimes are expected to impact ecosystem structure and function. These impacts may be determined by feedbacks between plants and associated microbes, including both endophytic fungal symbionts and viral pathogens. To test the role of biotic and abiotic factors in shaping virus prevalence in a managed grassland, we experimentally manipulated air temperature and precipitation, and after one growing season, evaluated four co-occurring grass species for infection by three species of barley and cereal yellow dwarf viruses (B/CYDVs). For one dominant grass species, tall fescue, we also manipulated fungal endophyte infection status, and tracked changes in viral prevalence over an additional two growing seasons for a total of three growing seasons. Plant species identity played a strong role in determining virus prevalence; Kentucky bluegrass and dallisgrass were more frequently infected than either tall fescue (endophyte-infected or endophyte-free) or Indian goosegrass. Both elevated temperature and elevated precipitation also had strong impacts on virus prevalence, but these effects varied among years and interacted with fungal endophyte symbiosis for tall fescue. In plots receiving additional precipitation in 2011, endophyte infection increased virus prevalence in tall fescue. Also in 2011, but

regardless of endophyte infection, elevated heat by itself increased virus prevalence for tall fescue. This effect of heat on virus prevalence may have been driven by vector population size because the viruses are obligately aphid-transmitted, and aphids were found more frequently in heated plots. Overall, our investigation suggests that both biotic and abiotic factors have important roles in disease dynamics in managed grasslands. Impacts of climate change on virus prevalence in grasslands may depend on the responses of aphid vectors, and the presence of endophytic fungal symbionts.

**Introduction**

Plants often interact with a diverse array of microorganisms, including enemies and mutualists (Gehring and Bennett 2009, Bennett 2010). For example, plant pathogens and mutualistic fungi are two classes of microorganisms that directly impact the plant and may in turn alter each other's success (Malinowski and Belesky 2006, Saikkonen et al. 2006). Furthermore, specific attributes of each species of microorganism may make them more or less likely to interact either directly or indirectly via their shared host or a third player such as an arthropod vector (Mitchell and Power 2006). Thus, a broad community context may be important for understanding microbial dynamics and their effects on community and ecosystem dynamics.

Here we consider the role of biotic and abiotic factors in shaping disease dynamics in a managed grassland. Grasslands cover just under half of the earth's land surface and provide multiple ecosystem services including: livestock production, maintenance of soil cover and biodiversity, as well as sequestration and storage of atmospheric $CO_2$ (Chakraborty 2001). Managed grasslands are often composed of a diverse group of forage species, including cool- and warm-season physiologies, sod-

forming or bunch growth forms, and grass, forb or legume functional groups (Burton and Hanna 1995). Such diverse species typically vary in their susceptibility and capacity to transmit generalist pathogens, which may lead to differences in the prevalence of virus infections among species and communities over space and time(Alexander 2010).

In addition to species identity and community composition, other biotic factors influencing may determine disease dynamics in managed grasslands. For example, fungal endophytes form symbiotic relationships with many plants. Fungal endophytes are estimated to occur in 20-30% of grass species (Leuchtmann 1992), across diverse ecological habitats (Vandenkoornhuyse et al. 2002), and on all continents with the exception of Antarctica (White 1994, Clay 1998). While the interaction between fungal endophytes and wild grass hosts in natural ecosystems can vary from mutualism to parasitism (Faeth and Fagan 2002, Saikkonen et al. 2010), the interactions of two important agricultural forage grasses, perennial rye grass (*Lolium perenne*) and tall fescue *(Schedonorus phoenix = Lolium arundinaceum = Festuca arundinacea*), with their Clavicipitaceous fungal endophytes are predominantly mutualistic (Clay and Schardl 2002). Specifically, endophytes enhance nutrient uptake (Malinowski et al. 2000, Newman 2003, Franzluebbers and Stuedemann 2005), increase drought tolerance (Arachevaleta et al. 1989, Malinowski and Belesky 2000), and provide protection from plant enemies including herbivores and some pathogens (Mahmood et al. 1993, Clay and Schardl 2002). These characteristics have led to widespread use of endophyte-infected plants in managed grasslands (Easton et al. 1994). Among herbivores, endophytes are particularly effective against aphids, reducing their feeding and abundance (Hartley and Gange 2009). Aphid population dynamics, host preference, and movement are pivotal to

the ecology of plant viruses (Power 2008). Arthropod vectors, particularly sucking insects like aphids, represent a majority of virus vectors and therefore their movement and host preference can control disease dynamics (Nault 1997, Hogenhout et al. 2008). Endophyte infection has negative effects on aphid preference and often decreases aphid fecundity (Hartley and Gange 2009), which may decrease pathogen transmission (Lehtonen et al. 2006). As such, endophytes are expected to play a key role in disease dynamics for managed grasslands.

In addition to these biotic factors, climate variability and change are likely to also influence disease dynamics. Changes in temperature and precipitation are expected to have impacts not only on plants (Luo et al. 2001, Knapp et al. 2002) but on plant diseases as well (Burdon et al. 2006). Climate change projections for the next 50-100 years in the southeastern U.S., and some other regions of the earth, predict that average annual rainfall may change by 10-30% and mean annual temperature will increase approximately 2ºC (IPCC 2007). Furthermore, minimum nighttime temperatures are projected to increase more than daytime maximum temperatures, and winter temperatures are projected to increase more than summer temperatures (Meehl et al. 2000, IPCC 2007). The seasonal nature of such changes in climate is likely to have serious direct effects for plant pathogens. As much as 99% of a pathogen population may be killed annually over the winter (Burdon and Elmqvist 1996); therefore, an increase in disease severity is expected as overwintering success of the pathogen increases with elevated winter temperatures (Harvell et al. 2002, Fabiszewski et al. 2010). Additionally, observed changes in vector overwintering and oversummering suggest that vector-transmitted diseases are also likely to have strong responses to changes in temperature (Bale et al.

2002, Newman 2003, Fabre et al. 2005, Garrett et al. 2006). Specifically, we expect increases in temperature to increase vector abundance, which will lead to an increase in the prevalence of vectored pathogens within the plant community (Fabre et al. 2005).

Climate change and endophyte infection may interact to control disease dynamics in managed grasslands. While there is still some variation in predictions, drought conditions are expected to change across regional scales (IPCC 2007). Under drought conditions, endophyte-infected tall fescue individuals generally replace endophyte-free individuals (Arachevaleta et al. 1989, Clay and Schardl 2002), suggesting that endophyte infection enhances drought tolerance. While the physiological mechanisms remain unclear, it has been suggested that endophytes alter the plant in several ways that may contribute to drought protection including: promoting deeper and denser roots, altering stomatal behavior, increased water tissue storage and enhanced osmotic adjustment in the meristem (Elmi and West 1995, Malinowski and Belesky 2000). Furthermore, endophytes may also increase alkaloid production when grown in drought conditions (Arachevaleta et al. 1989) and this increase in alkaloid concentration may act as osmotic protection for the plant (Schardl et al. 2004). This suggests that under drier conditions, such as frequently accompany higher temperatures (Arachevaleta et al. 1989), endophyte-infected plants may be more succulent but contain higher concentrations of anti-herbivore alkaloids than endophyte-free plants. It is currently unknown what the combined effect of these responses would be on arthropod herbivores and the pathogens they transmit. Decreased aphid abundance and virus prevalence on endophyte-infected plants could result from increased temperature if the effects of increased alkaloid production outweigh those of more succulent tissue on aphid preference and performance. Conversely, if the

effects of more succulent tissue outweigh those of increased alkaloid production, then increased temperature may increase aphid abundance and virus prevalence on endophyte-infected plants.

Here we present an experimental test of climate change drivers interacting with endophyte infection status to shape disease dynamics of a group of aphid-transmitted viruses in a managed grassland ecosystem. We manipulated temperature and precipitation regimes and tested the impacts on aphid abundances and the prevalence of virus infections. Additionally, we tested the roles that host identity and fungal endophytes play in disease dynamics.

## Materials and Methods

### *Study System*

Barley and cereal yellow dwarf viruses (B/CYDVs) are a group of viral pathogens that infect only grasses (Poaceae), including over 150 crop and noncrop species (D'Arcy 1995, Halbert and Voegtlin 1995). Infection is systemic through the phloem, where it causes necrosis and disruption of carbohydrate translocation (Irwin and Thresh 1990, D'Arcy 1995). Infection by B/CYDVs stunts plant growth (Malmstrom et al. 2005a), reduces root/shoot ratio (Kolb et al. 1991) and reduces longevity (D'Arcy and Burnett 1995). B/CYDVs are obligately transmitted by aphids.

Tall Fescue (*S. phoenix*) is a cool season grass that was introduced to North America and has been widely planted as a forage species due to its ability to tolerate high temperatures, drought conditions and grazing (Stuedemann and Hoveland 1988). Many of the properties that make *S. phoenix* attractive for use as a forage species can be attributed to its ability to form a symbiosis with the fungal endophyte *Neotyphodium*

*coenophialum* (Clay and Schardl 2002). It is estimated that 75-85% of *S. phoenix* in the U.S. is infected with *N. coenophialum* (Ball et al. 1993, Clay and Schardl 2002). This plant-fungus association provides a valuable model system for investigations of host-microbe-microbe interactions because they can build on previous studies of pair-wise host–fungus interactions and mechanisms for microbe-microbe competition (Saunders et al. 2010).

*Field Data*

This research project is located in a managed grassland or pasture at the University of Kentucky's Spindletop Agricultural Farm near Lexington, KY (Brosi 2011). Heat and precipitation were factorially manipulated among 20 plots divided into five blocks. All climate manipulations were based on the long-term average climate conditions of the site and future projections for the region. Plots designated as +precipitation received a 30% increase in long-term mean annual precipitation applied only during the growing season. Using a hexagonal array of infrared heaters mounted to posts and oriented toward the center of the hexagonal plots (two heaters per side), temperature was increased 3ºC over that of adjacent ambient plots, day and night, year-round for those plots designated as + heat. Treatments began in May 2009. In the spring of 2008, plots were seeded with a mixture of forage grass species including: tall fescue (*S. phoenix*) from the Kentucky 31 genotype planted at 50:50 common toxic endophyte-infected:endophyte-free, Bermuda grass (*Cynodon dactylon*) and Kentucky bluegrass (*Poa pratensis*). Naturally occurring crabgrass was also present.

Three times throughout the growing season, plots were mowed to remove aboveground biomass, simulating haying events. The diversity and richness of all plant

species were quantified at each mowing. From these initial species lists, four host species were identified for testing for B/CYDV infection because of their even distribution in all treatment plots. Samples of *P. pratensis*, *Eleusine indica* (Indian goosegrass) and *Paspalum dilatatum* (dallisgrass) were collected at the final mowing of the year on September 29, 2009. Individual leaves were harvested from random plants across the plots at 8cm from the soil surface. Samples were kept on ice in coolers and transported back to the lab. Virus infection status for *S. phoenix* (endophyte-infected and endophyte-free individuals) was assessed for three consecutive years using samples collected at the final mowing of each year (29 September 2009, 7 October 2010, 12 October 2011). Presence of the endophyte was determined using a monoclonal antibody test specific for *N. coenophialum* (Hiatt et al. 1999) in which plant material was assessed by blotting plant material onto Trans-Blot Transfer Medium pure nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA). While the tall fescue material was being tested for endophyte infection, the remaining plant material was stored in a -20ºC freezer at the McCulley Lab at the University of Kentucky. After endophyte infection status of individual tillers was determined, remaining plant material was sent overnight to the Mitchell Lab at the University of North Carolina at Chapel Hill. A compound indirect double-antibody sandwich Enzyme-linked Immunosorbent Assay (ELISA; Agdia Inc., Elkhart, IN, USA) was used on 0.1-0.3 g wet aboveground tissue to assay samples for the B/CYDV species BYDV-PAV, BYDV-MAV, and CYDV-RPV. Infection with one or more of these species indicates a positive infection status for the B/CYDV group.

Vector response to the climate treatments was assessed using field aphid surveys that were conducted in May 2010. Approximately twenty randomly chosen endophyte-

infected *S. phoenix* plants, and twenty randomly chosen endophyte-free *S. phoenix* and *P. pratensis* plants per experimental unit were searched for aphids (Appendix C2.1). Sample sizes for *S. phoenix* are slightly unbalanced because frequencies of endophyte-infected plants changed between 2009 and 2010, likely as a result of experimental manipulations (McCulley, *personal observation*).

*Data Analysis*

All data were analyzed using WinBUGS (Lunn et al. 2000) and R (v.2.13.1, R Foundation for Statistical Computing, Vienna Austria). The 'bugs' function within the 'arm' package was used to link the two programs (Gelman et al. 2012). Because treatments were applied to individual hexagons rather than individual plants, we used hexagon as a random effect in a mixed effects model to examine treatment effects on viral prevalence.

Using Bayesian methods, we fit a logistic regression with additive effects for treatment and species using a product Bernoulli (individual binary) model. The probability model for the data specifies a different probability model for each hexagonal experimental unit and has a binomial distribution. In the BUGS model the fixed effects portion of the model was:

$$logit(p) = \beta_0 + \beta_1 x_{1,i} + \ldots\ldots \beta_q x_{q,i}$$

where *x* variables are indicator variables for plot-level characteristics for all *q* regression coefficients. To specify the parameter portion of the model, the regression coefficients were given a normal prior, $\beta \sim N(0, 1.0e^{-5})$. In order to get the Markov chains tracking in the right area of the parameter space, we started with an informative prior ($\beta \sim N(0, 1.0e^{-3})$) for the intercept and intercept variance (for the first 1000 iterations). We then

switched to a BUGS model with uninformative priors, discarded the chains up until that point except for the last values which were used as starting values for the new chains, and then continued to fit the model.

We performed two sets of analyses in order to assess host species-level impacts on virus prevalence and long-term endophyte effects in tall fescue. In order to quantify host species-level impacts on virus prevalence, we used data collected in 2009 on four host species (dallisgrass, Indian goosegrass, Kentucky bluegrasss and endophyte-infected and endophyte-free tall fescue). To assess host-level impacts on virus prevalence, we fit all possible treatment and species combinations for dummy variables representing effects of '+Heat', '+Precipitation', 'Dallisgrass', 'Goosegrass', 'Bluegrass', and 'E+Fescue' (common toxic endophyte-infected vs. endophyte-free *S. phoenix*) using the samples collected in September 2009. To examine model fit, we generated 100,000 replicates of posterior predictive data for all data models. Posterior distributions were summarized by 95% Bayesian credible intervals (i.e. BCI; the 0.025 and 0.975 quantiles of the posterior distribution). Treatment combinations from the best model were then used to examine the probability of infection with individual viral species BYDV-PAV, BYDV-MAV and CYDV-RPV as well as the probability of co-infection with BYDV-PAV + BYDV-MAV, BYDV-PAV+CYDV-RPV and BYDV-MAV+CYDV-RPV. We focus on results with strong support, specifically those for which the 95% Bayesian credible intervals did not overlap zero.

In order to quantify long term impacts on virus prevalence we used data collected on endophyte-infected and endophyte-free tall fescue over a three year period (2009-2011). To assess long term impacts of the treatments on virus prevalence, we fit full

models using dummy variables for all possible treatments, endophyte status (free vs. infected) and sampling date (year) and their combinations. We tested effect on the probability of infection of tall fescue with one or more viral species (B/CYDV) as well as infection with individual viral species (BYDV-PAV, BYDV-MAV, CYDV-RPV). However, the relatively low prevalence of co-infection in tall fescue prevented us from testing effects on the probability of co-infection by multiple viral species.

The likelihood of aphid presence was also assessed. We fit all possible treatment and species combinations for dummy variables representing effects of '+Heat', '+Precipitation', 'Bluegrass', and 'PosFescue' (endophyte-infected *S. phoenix*) using the abundance data collected in May 2010. We examined model fits for aphid models in the same we examined model fits for virus prevalence models. We generated 100,000 replicates of posterior predictive data for all data models and posterior distributions were summarized by 95% Bayesian credible intervals.

For both the aphid data and the 2009 virus prevalence data, we fit a global model and a set of reduced models that embodied hypotheses about the effects of biotic and abiotic variables, then selected a best model for further analysis and inference based on deviance information criterion (DIC) statistics (Spiegelhalter et al. 2002). There were two models of 2009 overall virus prevalence with strong support: one that included dummy variables for all species, the heat treatment, the precipitation treatment and the interaction between precipitation and heat (DIC = 306.6; Appendix C2.2, Model 5), and another that was identical except for lacking the interaction between precipitation and heat (DIC=304.8; Appendix C2.2, Model 6). Because the primary goal of the experiment was to test the interaction between precipitation and heat, we proceeded with analyses using

the model that included the interaction. To allow direct comparison to effects on overall virus prevalence, we used models with the same explanatory variables to analyze effects on each virus species and on coinfections. While several models of aphid abundance had similarly strong support based on DIC (Appendix C2.2), Model 7 (including the factorial effects of temperature and precipitation, but no effect of endophyte) was the only model in which all of the effective sample sizes were considered adequate (equal to 1000), so we used this model as the best model. The 2009-2011 tall fescue data was fit with a global model.

**Results**

*Effects of Host Species Identity on Virus Prevalence:*

Host species differed strongly in their likelihood of infection by viruses at the virus group, individual virus species and co-infection levels (Table 4.1; Fig. 4.1). Across climate change treatments, the mean prevalence of virus infection was 26% for Kentucky bluegrass, 24% for dallisgrass, 16% for Indian goosegrass, 11% for endophyte-free fescue and 13% for endophyte-infected fescue (Appendix C1.1). Relative to endophyte-free fescue, dallisgrass was more likely to be infected by any virus, by BYDV-MAV, CYDV-RPV, and all three pairwise co-infections (Table 4.1). Kentucky bluegrass was more likely to be infected by any virus, by BYDV-MAV, CYDV-RPV, and by co-infections including CYDV-RPV (BYDV-PAV + CYDV-RPV and BYDV-MAV + CYDV-RPV; Table 4.1). Goosegrass was more likely to be infected by BYDV-MAV and by co-infections including BYDV-MAV (BYDV-MAV+BYDV-PAV and BYDV-MAV + BYDV-RPV; Table 4.1). Host species did not vary in likelihood of infection by BYDV-PAV, even though it was the most prevalent virus overall (Appendix C1.2).

Finally, across all hosts sampled in 2009, while neither the heat nor precipitation treatment by itself altered virus prevalence, the combination of elevated heat and precipitation decreased the likelihood of co-infection by BYDV-PAV and BYDV-MAV (Table 4.1).

*Effects of Endophyte Infection on Virus Prevalence in Tall Fescue:*

Increased heat and precipitation began to impact the prevalence of B/CYDV infection in tall fescue after three years of the experimental treatments, in 2011. In that year, elevated heat by itself increased the likelihood of virus infection in both endophyte-free and endophyte-infected tall fescue ($\hat{\beta}_{2011H}$ = 2.173, BIC = 0.087 to 4.59; Table 4.2). In the same year, in communities that received ambient heat and increased precipitation, endophyte infection increased virus prevalence ($\hat{\beta}_{2011PE+}$ = 14.6, BIC = 1.24 to 35.1; Table 4.2).

*Effects of Climate Drivers on Aphid Abundances:*

Increased temperature by itself increased the likelihood of aphids being present ($\hat{\beta}_{Heat}$ = 1.178, BIC = 0.031 to 2.436), while increased precipitation by itself ($\hat{\beta}_{Precipitation}$ = -0.433, BIC = -2.039 to 1.249), and the combination of increased precipitation and heat ($\hat{\beta}_{PrecipitationHeat}$ = -0.546, BIC = -2.572 to 1.301), did not influence the likelihood of aphids being present in a plot (Fig. 4.2; Appendix C2.1).

**Discussion**

Here we explored how biotic and abiotic factors impact disease dynamics in a managed grassland. We found strong effects of host species on the prevalence of three aphid-transmitted viruses. By the end of the experiment, elevated heat increased the prevalence of both aphids feeding on, and viruses infecting, the dominant grass species,

tall fescue. Also at the end of the experiment, precipitation regime modulated an effect of endophyte infection on virus prevalence in tall fescue, demonstrating the potential for interactions between biotic and abiotic factors.

Our results suggest that Kentucky bluegrass and dallisgrass may be important players for disease dynamics in managed grasslands. The odds of infection with one or more B/CYDV viral species were higher for Kentucky bluegrass and dallisgrass compared to tall fescue (endophyte-infected or endophyte-free) or Indian goosegrass. Furthermore, Kentucky bluegrass and dallisgrass were more likely to be infected with the less common viral species CYDV-RPV and BYDV-MAV, and (along with Indian goosegrass), more likely to be coinfected by CYDV-RPV and BYDV-MAV. Such differences in infection prevalence among host species could be a result of species specific characteristics which alter a host's ability to support vector populations, transmit infection to new hosts, and/or ability to become infected (LoGiudice et al. 2003, Borer et al. 2007, Cronin et al. 2010). These differences among host species can lead to effects of host community composition on virus prevalence (Borer et al. 2010, Delmiglio et al. 2010), suggesting that communities that include Kentucky bluegrass and dallisgrass will support greater virus prevalence. This effect could be magnified if the presence of these species increases virus transmission to other host species (Power and Mitchell 2004). Ultimately, such pathogen spillover could result in virus-mediated apparent competition, which may facilitate invasion of native grasslands (Malmstrom et al. 2005b, Malmstrom et al. 2007). Moreover, when host species vary in the degree to which they support pathogens, then effects of climate change on the relative abundance of host species may

have important consequences on pathogen transmission and diversity (Harvell et al. 2002, Fabiszewski et al. 2010).

Abiotic variables can also have a strong impact on viral disease dynamics in grasslands (Seabloom et al. 2009, Power et al. 2011). In a large-scale survey of the prevalence of B/CYDVs along a 2000-km latitudinal gradient in the western United States and Canada, prevalence declined with increasing precipitation (Seabloom et al. 2010). Our experimental manipulations of precipitation partially support this finding, as elevated precipitation had an overall negative effect on virus prevalence in tall fescue that became significant in the final year of the experimental treatments, except for endophyte-infected individuals. Plant mutualists such as foliar endophytes often positively affect plants when they are subjected to drought stress (Arachevaleta et al. 1989, Compant et al. 2010), but the response of such relationships to additional precipitation is not as well established. In a different study, elevated precipitation and endophyte infection interacted to increase concentrations of cellulose and hemicellulose in tall fescue (Brosi et al. 2011), which should decrease palatability to herbivores and possibly alter disease resistance (Vorwerk et al. 2004). Similarly, aphids tend to prefer plants with lower leaf percent carbon (Borer et al. 2009). Such changes in tissue chemistry may explain the increase in virus prevalence in endophyte-infected plants under increased precipitation. These results suggest endophyte infection may be an important player in determining disease dynamics under any future increases in precipitation.

Increased temperature may also alter disease dynamics in managed ecosystems. While some pathogens may be limited by increased temperature, others may benefit from decreased overwintering time or changes in vector behavior (Burdon et al. 2006, Garrett

et al. 2006, Fabiszewski et al. 2010). In our study, elevated temperature increased aphid presence, which translated to increased viral prevalence in the final year of the experiment, 2011. This supports previous field work with the aphid species *R. padi* that indicated increased temperatures in the spring and summer promotes the production of viruliferous aphids in the fall (Fabre et al. 2005). Theoretical results examining aphid response to increased temperature indicate increases in aphid presence and abundance due to increased temperature, but they also indicate that these populations exhibit complex dynamics that make them unstable over time (Zhou et al. 1997). This instability in aphid dynamics over time may lead to instability in disease dynamics over time. Surprisingly we did not see an interaction between endophyte infection and virus prevalence. This may be because the proportion of endophyte infected tall fescue decreased over time (Jim Nelson, *personal communication*) or because the effect of precipitation interacted with endophyte infection more strongly.

Changes in climate will not only result in areas that are hotter or wetter, but also areas with simultaneous increases in both temperature and precipitation (IPCC 2007). Predictions of viral disease dynamics for such situations are unclear as previous experimental manipulations often neglected this key component (Jones 2009). Here, the interaction of increasing temperature and precipitation did not change the odds of being infected with B/CYDV, perhaps simplifying predictions.

Overall, our field experiment revealed effects of abiotic factors, biotic factors and their interaction on disease dynamics in a managed ecosystem. The results suggest that impacts of climate change on virus prevalence in grasslands may depend on the responses of host species identity, aphid vectors, and the presence of endophytic fungal symbionts.

This complexity emphasizes the importance of considering multiple factors in the response of long-term disease dynamics to changes in temperature and precipitation regimes (Burdon et al. 2006, Alexander 2010).

**Acknowledgements**

We thank Glade Brosi and Jim Nelson at the University of Kentucky for help with aphid surveys and technical support. We are grateful to Jack Weiss for statistical advice. We would also like to thank the Mitchell lab for assistance. This research was partially supported by the joint NSF-NIH Ecology of Infectious Disease program through NSF Grants EF-05-25641 and DEB-10-15909 to C.E.M., Kentucky Agricultural Experiment Station funds (KY006045) and DOE-NICCR grant DE-FC02-06ER64156 to R.L.M. and an NSF GRFP to M.A.R.

**Figure 4.1. Bayesian credible intervals for the log odd ratio of virus infection.** Bayesian credible intervals for the log odds ratio of virus infection in three host species and endophyte-infected tall fescue, relative to endophyte-free tall fescue (the denominator of each odds ratio) from a Markov chain Monte Carlo simulation describing B/CYDV infection. The likelihood of virus infection was greater for Kentucky bluegrass and dallisgrass. The red-dotted line indicates equal odds of infection, and the red 'x' indicates observed means using a frequentist model.

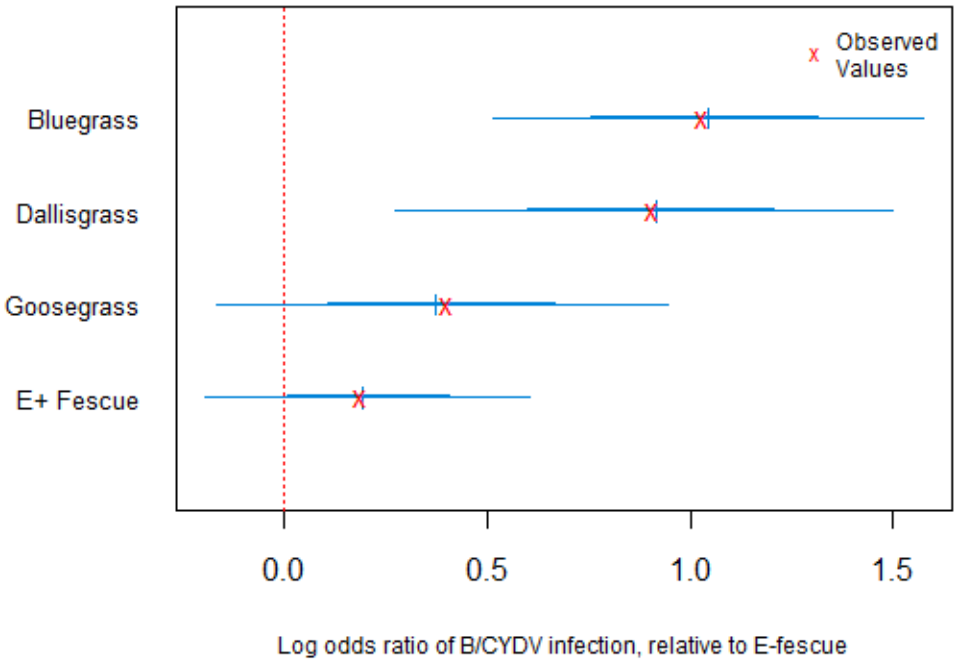


**Figure 4.2. Bayesian credible intervals for the log odds ratio of aphid presence.** Bayesian credible intervals for the effects of elevated heat ('H'), elevated precipitation ('P'), and elevated heat and precipitation ('HP') on aphid presence, relative to ambient heat and precipitation. Increased heat by itself increased the log odds ratio of aphids being present. The red-dotted line indicates equal odds of infection, and the red 'x' indicates observed means using a frequentist model.

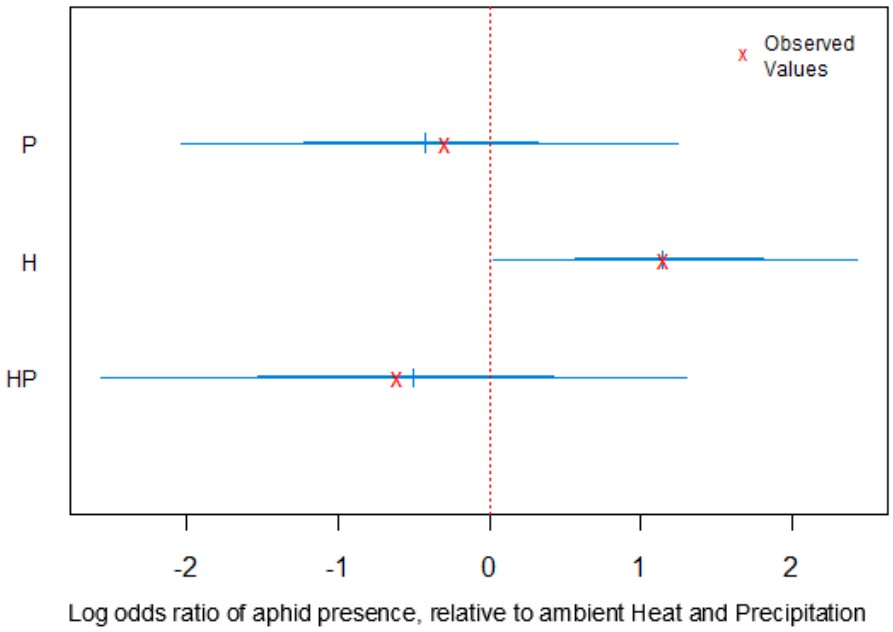


**Table 4.1. Parameter estimates from the binomial model describing virus infection in 2009.** Parameter estimates (marginal posterior means) from the binomial model for the effects of increased Heat, increased Precipitation ('Precip'), their interaction, and host species on the log odds ratio of virus infection, relative to endophyte-free *Schedonorus phoenix* or to ambient heat and precipitation. Heated plots received an increase in temperature of 3ºC, day and night, year-round. Precipitation ('Precip') plots received a 30% increase in long-term mean annual precipitation applied during the growing season. Plots designated as 'Heat x Precip' received both treatments. Species include "Bluegrass" (*Poa pratensis*), "Dallisgrass" (*Paspalum dilatatum)*, "Goosegrass" (*Eleusine indica)* and "E+ Fescue" (endophyte-infected *Schedonorus phoenix)*. 95% Bayesian credible intervals (i.e. the 0.025 and 0.975 quantiles of the posterior distribution) are shown in parentheses below each parameter estimate; bold font indicates those that do not cover zero.

| | **Heat** | **Precip** | **Heat x Precip** | **Bluegrass** | **Dallisgrass** | **Goosegrass** | **E+ Fescue** |
|---|---|---|---|---|---|---|---|
| **B/CYDVs** | -0.2 | -0.4 | 0.2 | **1.0** | **0.9** | 0.4 | 0.2 |
| | (-0.7 to 0.3) | (-0.9 to 0.1) | (-0.6 to 1.0) | **(0.5 to 1.6)** | **(0.3 to 1.5)** | (-0.2 to 0.9) | (-0.2 to 0.6) |
| ***Individual Virus Species*** | | | | | | | |
| **PAV** | -0.3 | -0.3 | 0.0 | -0.1 | 0.3 | -0.2 | 0.3 |
| | (-1.0 to 0.4) | (-1.0 to 0.4) | (-0.9 to 1.0) | (-0.9 to 0.7) | (-0.5 to 1.0) | (-0.9 to 0.5) | (-0.2 to 0.7) |
| **MAV** | -0.1 | 0.0 | -0.3 | **5.3** | **5.5** | **5.3** | 1.6 |
| | (-1.1 to 0.9) | (-1.0 to 1.0) | (-1.7 to 1.0) | **(3.1 to 8.9)** | **(3.3 to 9.1)** | **(3.1 to 8.8)** | (-1.1 to 5.2) |
| **RPV** | -0.2 | -0.7 | 0.6 | **1.0** | **1.9** | 0.3 | 0.1 |
| | (-1.0 to 0.6) | (-1.6 to 0.1) | (-0.7 to 1.8) | **(0.1 to 1.8)** | **(1.1 to 2.7)** | (-0.7 to 1.2) | (-0.7 to 0.8) |
| ***Co-Infections*** | | | | | | | |
| **PAV+RPV** | -1.4 | -0.3 | 1.3 | **-24.1** | **3.3** | 1.0 | 0.7 |
| | (-3.7 to 0.5) | (-2.0 to 1.0) | (-1.3 to 4.1) | **(-67.7 to -0.2)** | **(2.0 to 5.1)** | (-1.2 to 3.1) | (-0.9 to 2.6) |
| **PAV+MAV** | -1.1 | -0.4 | **-25.7** | -23.5 | **4.8** | **4.2** | 1.7 |
| | (-2.9 to 0.4) | (-1.8 to 1.0) | **(-71.2 to -1.4)** | (-67.8 to 1.5) | **(2.6 to 7.9)** | **(2.0 to 7.5)** | (-1.0 to 4.9) |
| **MAV+RPV** | 0.0 | 0.0 | -0.8 | **3.8** | **4.9** | **3.7** | 0.5 |
| | (-1.7 to 1.5) | (-1.5 to 1.5) | (-3.5 to 1.6) | **(1.5 to 7.0)** | **(2.8 to 7.8)** | **(1.5 to 6.8)** | (-3.2 to 4.1) |

**Table 4.2. Parameter estimates from the binomial model describing *Schedonorus phoenix* virus infection 2009-2011.** Parameter estimates (marginal posterior means) from the binomial model for the effects of heat ('H'), precipitation ('P'), year, and endophyte infection ('E+') on the log odds ratio of virus infection, relative to endophyte-free *Schedonorus phoenix* or to ambient heat and precipitation or to 2009. Parameter estimates and 95% BCIs are shown for each treatment combination; bold font indicates those that do not cover zero.

| Treatment | Parameter Estimate | BCI |
|---|---|---|
| *P* | -0.302 | (-1.24 to 0.624) |
| ***H*** | **-1.08** | **(-2.11 to -0.096)** |
| *E+* | 0.026 | (-0.75 to 0.769) |
| *2010* | 0.15 | (-0.671 to 0.966) |
| *2011* | -0.489 | (-2.50 to 1.04) |
| *HP* | 0.857 | (-0.558 to 2.33) |
| *P E+* | 0.061 | (-1.05 to 1.18) |
| *H E+* | 0.731 | (-0.424 to 1.96) |
| *2010P* | -0.132 | (-1.46 to 1.15) |
| ***2011P*** | **-13.2** | **(-33.5 to -0.549)** |
| *2010H* | 0.813 | (-0.428 to 2.16) |
| ***2011H*** | **2.173** | **(0.087 to 4.59)** |
| *2010 E+* | -0.053 | (-1.27 to 1.13) |
| *2011 E+* | -1.1 | (-4.62 to 1.72) |
| *HP E+* | -0.758 | (-2.40 to 0.941) |
| *2010HP* | -1.23 | (-3.42 to 0.713) |
| *2011HP* | 11.6 | (-1.61 to 32.2) |
| *2010P E+* | -0.265 | (-2.27 to 1.63) |
| ***2011P E+*** | **14.6** | **(1.24 to 35.1)** |
| *2010H E+* | -1.33 | (-3.48 to 0.674) |
| *2011H E+* | 0.344 | (-3.36 to 4.50) |
| *2010HP E+* | 2.01 | (-1 to 4.98) |
| *2011HP E+* | -13.2 | (-33.4 to 0.746) |

# CHAPTER V: THE EFFECT OF MUTUALISTS ON PATHOGEN-HOST DYNAMICS


## Abstract

Theoretical explorations of interspecific species interactions have traditionally been studied from a pairwise point of view. This has led to the development of an extensive body of theory on both mutualisms and disease, but neglects multiple species interacting with the same host at the same time. We developed a model of the interactions among a fungal mutualist, a viral pathogen and their shared plant host, which we parameterized using a greenhouse experiment. Both microbes were assumed to alter the uptake and use of soil nutrients by the plant. We found that the productivity of the system and the strength of the plant-fungal mutualism influenced community dynamics. In particular, plants are obligately dependent on their mutualist to forage for soil resources at low resource availability and facultatively dependent on their mutualist at high resource availability. If the fungus is not a sufficiently effective mutualistic partner, then the pathogen drives the host extinct before the fungus can fully establish. Further, the natural enemy can both depend on the presence of the fungal mutualist for persistence and cause it to go extinct. We observed cyclic plant-enemy population dynamics. Specifically, the enemy causes instability in the model such that it drives the plant below a threshold where the enemy can persist, which allows the plant and fungus populations to rebound, which then allows the enemy to re-invade and repeat the cycle. Thus, the

natural enemy can both depend on the presence of the fungal mutualist for persistence and cause it to go extinct. Further, these results indicate that the microbes may interact to facilitate or inhibit one another. In total, association with mutualists can alter host-enemy interactions, and the reverse is also true in that enemies may alter host-mutualist interactions.

**Introduction**

Interspecific species interactions can take a variety of forms. Often such interactions vary in space and time. They also differ both quantitatively and qualitatively, including from mutualistic, in which both species benefit, to parasitism, in which one species receives a net benefit and the other receives a net detriment. Historically, ecologists have thought about such interactions as pairwise with one symbiont and one host; however, recent research has begun to consider multispecies interactions as they co-occur within an ecological community (Bruno et al. 2003, Gehring and Bennett 2009, Bennett 2010). Therefore, research that considers multispecies interactions is the next step towards understanding ecological communities (Strauss and Irwin 2004, Morris et al. 2007, Van Der Putten 2009).

The influence of multispecies interactions on host development, growth and performance has been an area of developing interest in both terrestrial and aquatic ecosystems (Bronstein and Barbosa 2002, Bruno et al. 2003, Little and Currie 2009). In three-species systems, the third player can alter the intensity, outcome and even the symbiotic state (mutualistic or parasitic) of an association (Bronstein and Barbosa 2002). For example, specialized microfungal parasites that infect fungal gardens which are

cultivated by ants as an obligate mutualist can stabilize cooperation between the ants and the cultivated fungus and thus keep the mutualism honest (Little and Currie 2009).

Plant hosts provide an ecologically important arena with which to examine multispecies interactions. For example, plant phenotypes can be fundamentally altered by microbes that influence biochemical pathways and provide plants access to microbial biochemical pathways, impacting both host nutrition and defense against natural enemies (Friesen et al.). Additionally, plants are experimentally tractable and thus experimental manipulations of multispecies interactions are possible (i.e., Chapter 2, 3 of this dissertation).

One key determinant of the outcome of interactions among diverse organisms within host plants appears to be the location of each interactor. While there is still much variation in such interactions, in general, aboveground organisms tend to negatively impact belowground organisms but the reverse is not always true (van Dam and Heil 2011). Specifically, aboveground herbivores generally reduce the performance of belowground organisms, but belowground herbivores more often facilitate feeding of aboveground herbivores (van Dam and Heil 2011).

Furthermore, the effects of enemy damage on plant performance often depend on the identity of the plant mutualist. Fungal mutualists such as foliar endophytic fungi and mycorrhizal fungi can have differential impacts on plant hosts and their enemies. Sucking insects such as aphids are often negatively affected by endophytes but respond positively to mycorrhizae, and leaf-chewers tend to be negatively affected by both types of fungi (Hartley and Gange 2009, Koricheva et al. 2009). Additionally, there is variation in effects of fungal mutualists on plant tolerance to enemies within a fungal type. Different

mycorrhizal fungal species can increase, decrease, or have no effect on plant tolerance to herbivory (Bennett and Bever 2007). Theoretical models predict improved nutrition and increased tolerance in plants associating with mycorrhizal fungi will result in larger enemy populations (Bennett et al. 2006). Empirical evidence to test this prediction with actual herbivore or pathogen enemies is lacking; however, research exploring the interaction between a pathogen and mycorrhizal fungi under elevated CO2 indicates, mycorrhizal association increased the titer of virus infections, and virus infection reciprocally increased the colonization of roots by mycorrhizal hyphae (Chapter 2, this dissertation). This suggests that this mutualist and pathogenic organism interact to alter each other's success.

Conversely, the presence of mutualists may lessen the negative effect of enemies on plant performance (Morris et al. 2007) Chapter 3, this dissertation). A possible mechanism for this could be that mutualists induce plant chemical defenses (Friesen et al. 2011). Belowground organisms commonly induce defense responses that extend aboveground and vice versa (Bezemer and van Dam 2005). On the other hand, plant tolerance to herbivore enemies can be negatively correlated with mycorrhizal fungal hyphal density, which suggests that herbivory can reduce mycorrhizal fungal benefits by decreasing hyphae (Garrido et al. 2010).

Furthermore, enemy identity can also influence the outcome of plant-enemy-mutualist interactions. The impact of interactions of mycorrhizal fungi with either belowground pathogens or aboveground herbivores on plant performance depended upon the specific type of enemy examined (Borowicz 2001, Gehring and Whitham 2002). Microbial pathogens are one type of enemy whose impact on agroecosystems is well

known (Mitchell and Power 2006), but their influence on natural community dynamics is not as well understood (Alexander 2010, Rúa et al. 2011). Recent efforts have been made to incorporate pathogens into ecological community research. For example, experimental removal of foliar fungal pathogens in a UK grassland revealed that fungi significantly reduced aboveground plant biomass and promoted plant diversity (Allan et al. 2010). Furthermore, the impact of pathogen infection on the host plant can vary widely because host plants vary in resistance and tolerance to infection (Barrett et al. 2009). Since microbial mutualists have been known to alter plant physiological traits associated with pathogen resistance and tolerance (Friesen et al. 2011), the association of a mutualist may be one way in which a host can increase its tolerance to pathogen attack (Bennett et al. 2006).

While experiments suggest that pathogen-host-mutualist interactions have the potential to create system dynamics, only a few theoretical studies have explored these interactions. Bennett et al. (2006) examined how effects of a mutualist on the values of the parameters in the Rosenzweig and MacArthur (1963) consumer-resource model alter the stability and equilibrium abundance of plant and enemies. We extended this model to allow the presence of enemies to alter the plant-fungal interaction and to consider changes in the host-enemy-mutualist framework over time.

**Model Description and Analyses**

To more fully explore the relationship between microbial abundances and host responses, we developed and analyzed a model of interactions between populations of a plant host, a pathogen and a mutualist. The model assumes that both pathogen and mutualist have direct impact on the ability of plants to take up a single limiting nutrient.

Appendix D describes the full model. Here, we describe the non-dimensionalized version that we analyzed. The model of three differential equations (1) describing the changes in abundances of the plant host (H) , its fungal mutualist (M) and microbial enemy (P) .

$$\frac{dP}{dt} = H \cdot c \left(\frac{P}{1+H \cdot b}\right) - (d \cdot P)$$

$$\frac{dH}{dt} = i \cdot H \left(\frac{1+k \cdot M}{1+P}\right)(n - H) - (f \cdot H) \qquad (1)$$

$$\frac{dM}{dt} = H\left(\frac{M}{1+M}\right) - M$$

The model reflects the following set of simple assumptions about the effects of the environment and interactions on the population growth rate. All three species have a constant loss rate. The plant population is limited by a single limiting nutrient that is not shared by the other two species and is instantaneously recycled into the environment. The uptake rate of this nutrient is increased in a linear fashion with the abundance of the mutualist and declines as an inverse of the pathogen. The pathogen population increases as an increasing function of plant abundance that saturates with increasing pathogen populations. The mutualist population also increases with increasing plant abundance and their benefit saturates with increase plant abundance. We note that neither microbe directly alters the growth rate of the other. Since our model is primarily concerned with the interaction of a fungal mutualist and a plant pathogen, throughout the remainder of our model analyses we will refer to the mutualist as the fungus or fungal mutualist and the microbial enemy as the pathogen.

Nullsurface analysis was used to determine general model behavior. Invasion criteria can inferred in some cases by taking the plant-fungal mutualist phase plane and projecting the nullsurfaces onto it (Umbanhowar and McCann 2005). This is made possible because nullsurfaces of both fungi and enemy do not vary with changes in

enemy density so these surfaces are constant as enemy density changes. These analyses demonstrate several qualitatively different and biologically important outcomes from the model.

These outcomes can be categorized in several ways. First, the plant-mutualist interaction can have one or two non-trivial positive equilibria. In the case of one equilibria, the equilibrium is stable. In the case there are two equilbria, there is one stable equilibrium and one saddle. In this situation, when plant and fungal initial populations are below a set of abundances (a separatrix) the plant-fungal populations will tend towards a lower, trivial equilibrium with either both species extinct, or where plants are present in the absence of mutualist. In the single nontrivial equilibrium, three alignments of the pathogen nullcline represent three different biological interesting outcomes: pathogen-induced exclusion of mutualists, three-species coexistence, and mutualist-enabled pathogen persistence (Figure 5.1A). In the presences of threshold behavior, similar outcomes exist, but in this case, the presence of pathogens can drive the plant and mutualists to abundances below the separatrix. This leads to two biologically interesting outcomes—one where pathogens require mutualists in order to be present in the system while simultaneously driving plant populations below threshold abundances sufficient to maintain its own population. Second, if the plant is a truly obligate mutualist, the pathogen can drive all three species extinct.

**Numerical analysis**

Invasion criteria are not sufficient, by themselves, to demonstrate the global persistence of the interacting populations and the stability of the equilibria. To further explore qualitative changes in the behavior of our model, we used numerical simulation

with explicit parameter estimates. The model was parameterized with values derived from a greenhouse experiment (Appendix D) and literature searches when necessary (Table 5.1A, 5.1B). Additional parameter analyses were completed to determine the effect of different ranges in parameter values on host, pathogen and mutualist abundances. All analyses were completed in MATLAB R2010b (MathWorks, Natick, MA) or with the 'vode' function of the 'deSolve' package (Soetaert et al. 2011) in R (v.2.13.0, R Foundation for Statistical Computing, Vienna Austria).

A nutrient threshold exists that determines the relationship between the host and the fungal mutualist, and consequently the stability of the system. At nutrient values less than this threshold, the mutualism is obligate for the plant, and the enemy increases in abundance, which drives the host and mutualist below their threshold abundances, leading to stability of the system (Fig. 5.2A). In contrast, when the nutrient parameter ($n$) is set equal to a relatively high value of 100, nullcline analysis suggests, and numerical simulation confirms, that pathogen persistence is facilitated by the presence of the mutualist. In this situation, the fungal mutualist increases the host equilibrium abundance enough that the pathogen can invade. At nutrient values greater than this threshold, the association with the mutualist is facultative for the plant, which allows the enemy to persist and results in cycling of the three species model (Fig. 5.2B).

Varying the strength of the mutualism and enemy effects further changes equilibrium responses of the model. When the effective rate of the fungus ($k$) is increased, stable limit cycles occur under both facultative (high $n$) and obligate (low $n$) mutualistic situations. Only the enemy increases in amplitude but the minimum population abundances also change when the mutualist is facultative (Fig. 5.2B). However, across the range of fungus effective rates, at high nutrient values the plant-enemy cycles drive the plant to low enough levels that the fungus is excluded but

is able to recover (Fig. 5.3C,F). Nullcline analysis at a high and intermediate nutrient value support these dynamics (Fig. 5.3,B,C). When the mutualist is obligate at low nutrient values, there are no cycles (Fig. 5.3A) which is also demonstrated with numerical integration (Fig. 5.2A).

The effect of enemy efficiency (*c*) on stability is similar to that of nutrient inputs. High values of either parameter destabilize the system and thereby prevent three-species coexistence, echoing the paradox of enrichment (Rosenzweig 1971). At low enemy efficiencies and as nutrient supply is increased, the outcome shifts from a situation in which the enemy is excluded because plant densities are too low to support the presence of the pathogen (Fig. 5.4A, Fig.5.5A) to three-species coexistence facilitated by the presence of the fungus (Fig. 5.4B), and eventually to host extinction due to an overabundance of pathogen that drives the host extinct before the fungus can invade (Fig. 5.4C, Fig. 5.5B). At high enemy efficiencies and as nutrient supply is increased, the outcome shifts from enemy exclusion because of low plant densities (Fig. 5.4D) to host extinction due to high pathogen abundance (Fig. 5.4E,F), and intermediate nutrient supply rates do not allow three-species coexistence (e.g. Fig. 5.4E).

**Discussion**

This paper has explored the equilibrium dynamics surrounding the interspecific interaction of two plant microbes and their host. Additionally, it has explored the threshold dynamics surrounding different parameter values. We found that pathogen persistence can be facilitated by the presence of the fungal mutualist. Moreover, the type of mutualistic association (facultative vs. obligate) was very important for enemy persistence and stability. For our model, the type of mutualism is determined by a nutrient threshold such that below it the mutualist-host relationship is obligate while above the threshold the mutualist-host relationship is facultative. This shift in relationship

changes the host-pathogen dynamics such that the negative effect of the pathogen decreases with increasing nutrients. Such shifts in dynamics indicate that microbes may facilitate or inhibit one another.

One way in which microbes may appear to inhibit one another but actually facilitate one another's existence can be viewed through theoretical work on exploiters in mutualistic relationships. Symbiotic relationships in which one species cheats a mutualist of potential benefits without reciprocating have often been considered destabilizing to mutualisms (Bronstein 2001). Using a continuous-time model of the yucca–yucca moth system involving plants, pollinating seed parasites and nonpollinating seed parasites, Morris et al. (2003) demonstrated a mutualist-exploiter relationship such that competition plays an important role in three-species co-existence. Furthermore, exploiters can invade the stable mutualism and coexist with mutualists in the presence of weak intra- and interspecific competition (Morris et al. 2003). Long–term persistence of mutualisms in which a cheater is present is also possible when there is asymmetrical competition within species for the commodities (resources) offered by mutualistic partners but the cheater will drive itself and consequently the system extinct if there is no cost for the cheater (Ferriere et al. 2002). Our model demonstrates similar dynamics in that for co-existence to occur, a series of negative feedbacks must occur in which the enemy drives itself extinct, allowing for the rebound of the plant and mutualist populations, followed by reinvasion of the enemy. One of the limitations of our model is that it is an ecological model and does not consider the potential for evolution to change the interaction parameters; however, the existence of threshold dynamics is a good

indicator of the limits in this system which may prevent long-term persistence or co-existence.

Due to the complexity of mutualisms and pathogenic relationships, the existence of threshold dynamics is not surprising. As previous models with exploiters have indicated, division of resource use such that both partners exchange commodities at levels which satisfy each other without damaging their own success is important for long-term model persistence (Ferriere et al. 2002). We demonstrate the importance of nutrient thresholds for determining the type of mutualistic association. We have assumed that microbes only interact via changes in host abundance. However, these microbes may interact more directly by changing host quality. For example, by altering host nutrition, a mutualist may stimulate enemy population growth (Bennett et al. 2006). This may be particularly true for viral pathogens which can be phosphorus limited if the host has a high C:P ratio (Clasen and Elser 2007). Mycorrhizal fungi are ubiquitous plant symbionts that play an important role in the acquisition of less mobile mineral nutrients, particularly phosphorus (Smith and Read 2008). In return, mycorrhizal fungi receive carbohydrates from the plant. In addition to altering leaf level photosynthesis (Smith and Read 2008), mycorrhizal fungi can increase plant root growth (Bryla and Eissenstat 2005). Since mycorrhizal fungi increase plant phosphorus (Tang et al. 2006, Smith and Read 2008), it is possible that host plants associating with mycorrhizal fungi may have higher viral pathogen loads due to the higher shoot phosphorus content. Thus, such increases in plant phosphorus due to mycorrhizal association may allow the plant to support a higher viral titer load that if viruses are phosphorus-limited (Clasen and Elser 2007, Borer et al. 2010). There is some evidence in agricultural systems which show an increase in viral

titer as a result of association with mycorrhizae (Daft and Okusanya 1973, Schonbeck 1979), but such reports are limited.

In addition to resource partitioning, the spatial structure of multispecies interactions is important for co-existence. Currently, in addition to not being explicit about nutrient flow through the system, our model is spatially homogenous which limits our understanding of the exact infection structure. Experimental evidence with multiple mutualistic fungi indicates that host plants are able to preferentially allocate resources to beneficial fungi, which can overcome the cost of the mutualism and allow the beneficial fungi to increase (Bever et al. 2008). The same may be true for host-pathogen-mutualist relationships where the host may preferentially allocate to the mutualist as a way to overcome the negative effects of the pathogen, although empirical evidence considering such possibilities is lacking.

The model we analyzed is a mean-field model that assumes that no spatial structure in plant-microbe associations. This approach conflates the process of transmission and within host growth for both the fungal mutualist and natural enemy. In real populations of plants, individual plants may be infected or not by both mutualistic fungi and pathogens. All three could potentially have different ability to disperse, leading to spatial structure in the amount and extent of cross infectivity. Molofsky et al (2001) showed how limited dispersal can have dramatic effects on population dynamics where threshold behaviors are present; however analyses of spatial structure are beyond the scope of the current analysis. These results have important implications for microbe host dynamics. Despite their common occurrence, such microbe-microbe interactions are often neglected. We demonstrate that a series of negative feedbacks as a result of host-

enemy dynamics create instability in the host-mutualist model, leading to instability in the full model, suggesting the importance of such three-way interactions for understanding community processes.

**Acknowledgements**

We would like to the Mitchell Lab for discussions. This research was partially supported by the joint NSF-NIH Ecology of Infectious Disease program through NSF Grants EF-05-25641 and DEB-10-15909 to C.E.M. and an NSF GRFP to M.A.R.

**Figure 5.1. Isocline analysis for our model.** To understand how mycorrhizal fungi might alter population dynamics of host-pathogen relationships, we modified the Lotka-Volterra predator-prey equations to reflect host-pathogen dynamics. Next we graphed the isoclines of these equations (red=plant, black=mutualist, blue=enemy), and total nutrient availability (in green). In *A*, we have a situation in which the enemy is excluded because plant densities are too low to support the presence of the pathogen. In *B*, we obtain three species co-existence. The pathogen keeps the plant at low abundances but the fungus can still invade. In *C* the pathogen can drive the plant to low enough levels that the fungus cannot invade. Finally, in *D* we have three species coexistence such that pathogen existence is facilitated by the presence of the fungi. Specifically the fungi increase the threshold of the enemy enough so that the enemy can invade. The increasing fungus threshold increases plant abundance which increases the carrying capacity of the plant for the pathogen (essentially how much enemy the plant can support) which finally increases the pathogen threshold for the system.

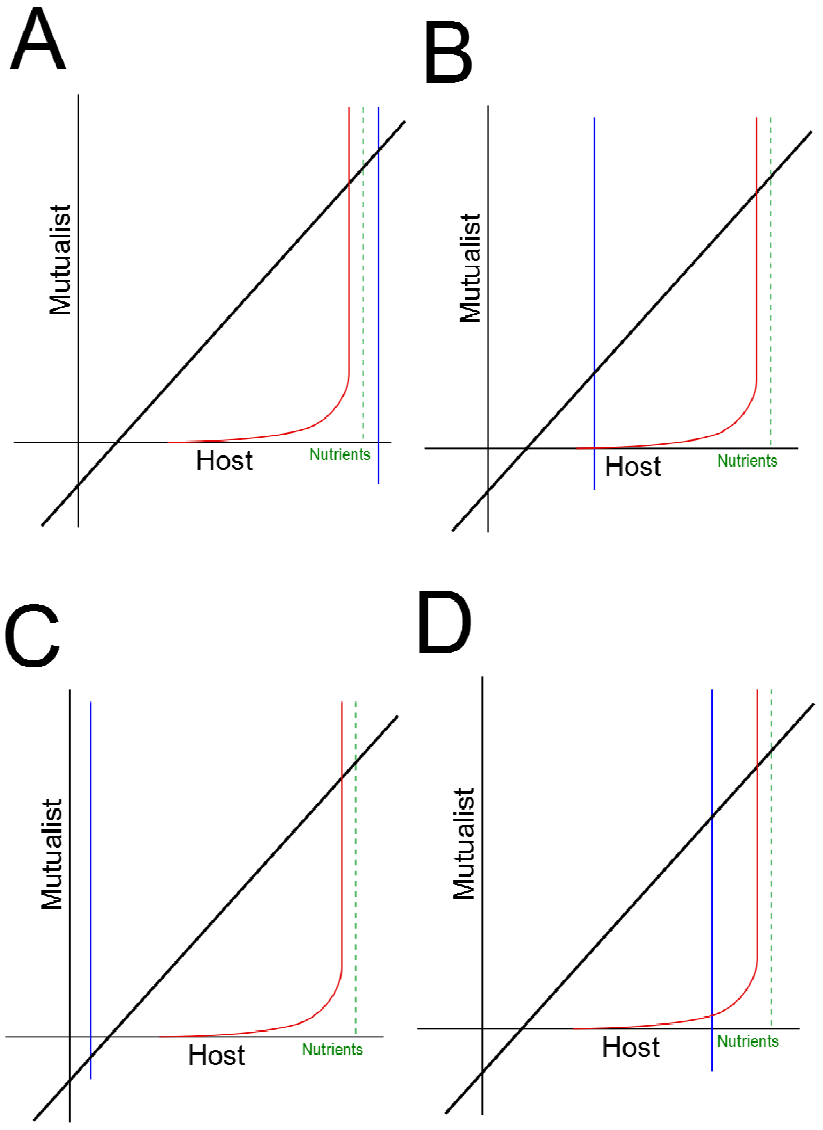


**Figure 5.2. Numerical integration across the plant-mutualist nullcline space, constant *k*.** Numerical integration across the plant-mutualist nullcline space simulated over time under varying nutrient inputs at a constant fungal effective rate (k=0.154). These dynamics inform the nullcline dynamics demonstrated in Figure 5.3 A,C. Nutrient rates increase from N=25 (obligate mutualist, panel A) and N=100 (facultative mutualist, panel B). In order to ensure equilibrium dynamics, a 300 step time transient was run and the following 300 steps are graphed below.

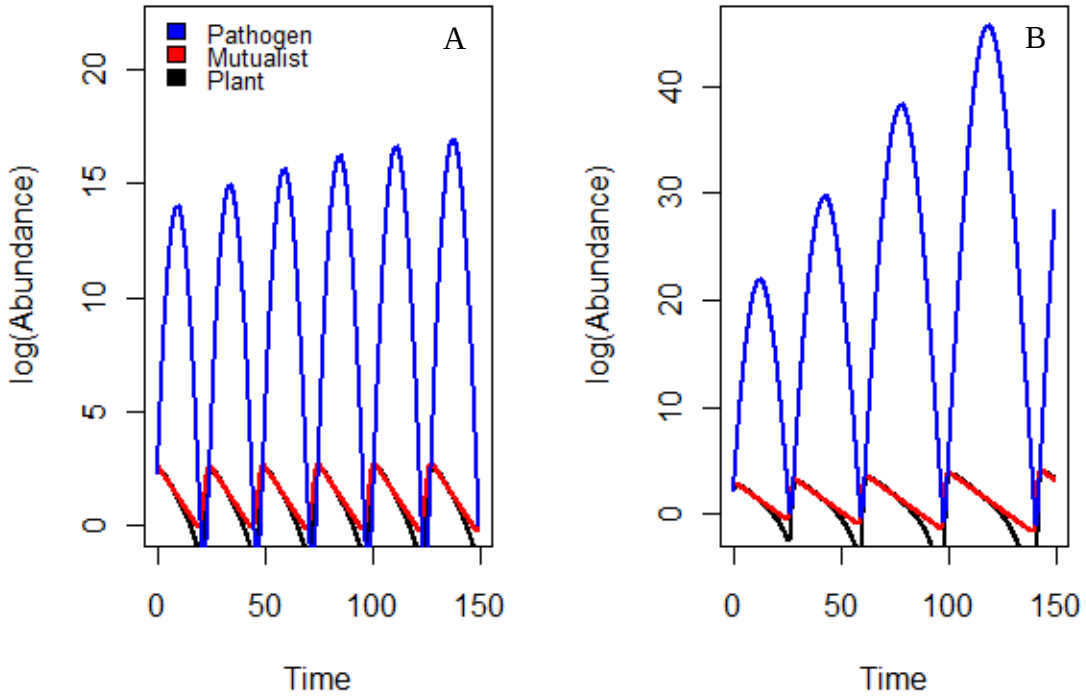


**Figure 5.3. Nullclines for the enemy graphed on the plant-mutualist nullcline space, varying *k*.** Nullclines for the enemy graphed on the plant-mutualist nullcline space when you alter the strength of the plant-mutualist relationship (*k*) and nutrient input (*N*). Panels A-C represent nullclines of increasing nutrient inputs at a low fungal effective rate (k=0.2) and panels D-F represent nullclines of increasing nutrient inputs at high fungal effect rates (k=0.75).

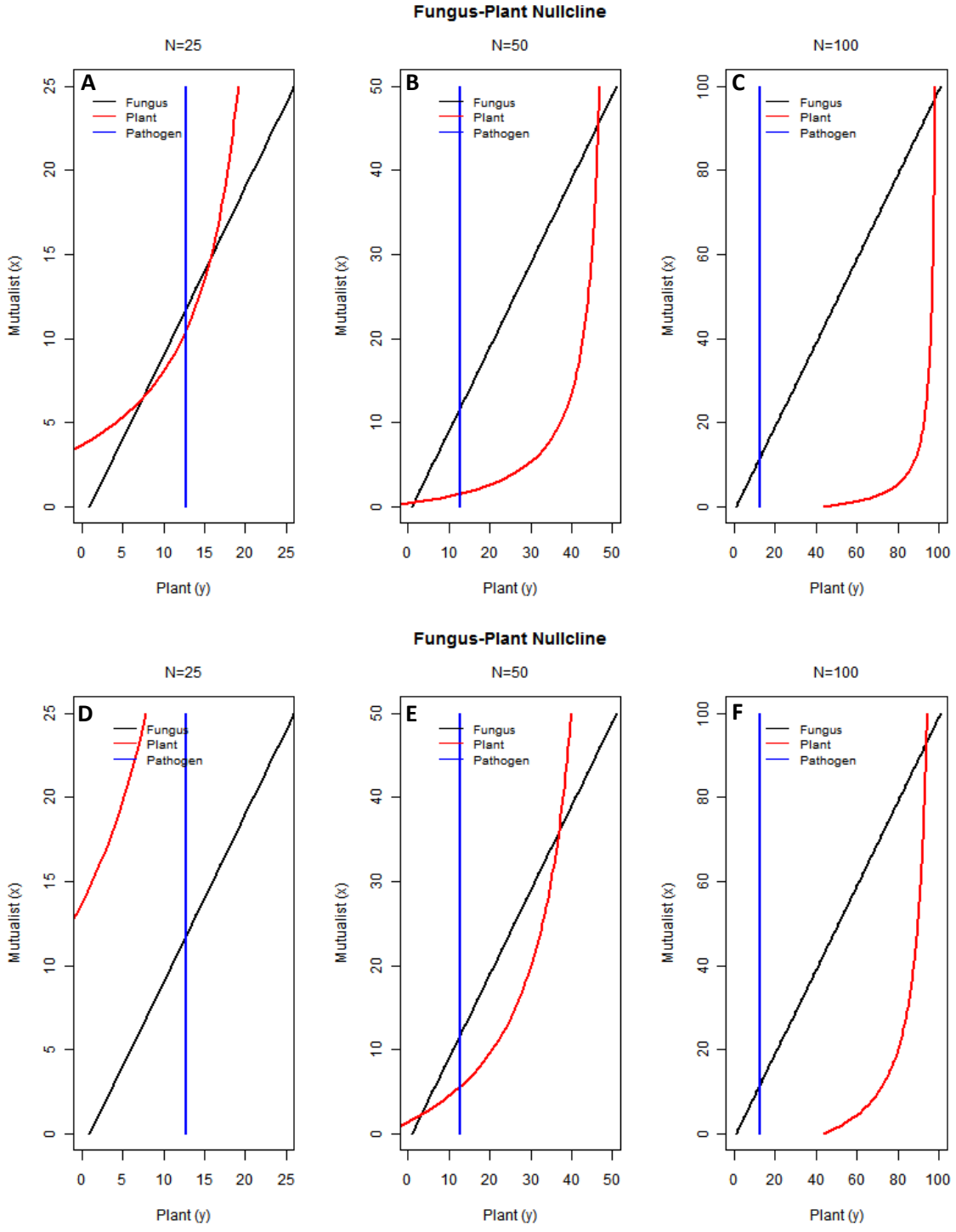


**Figure 5.4. Nullclines for the enemy graphed on the plant-mutualist nullcline, varying *c*.** Nullclines for the enemy graphed on the plant-mutualist nullcline space under varying strength of the plant-enemy relationship (*c*) and nutrient input (*N*). Panels A-C represent nullclines of increasing nutrient inputs at a low enemy efficiency (c=0.2) and panels D-F represent nullclines of increasing nutrient inputs at high enemy efficiency (c=1.75).

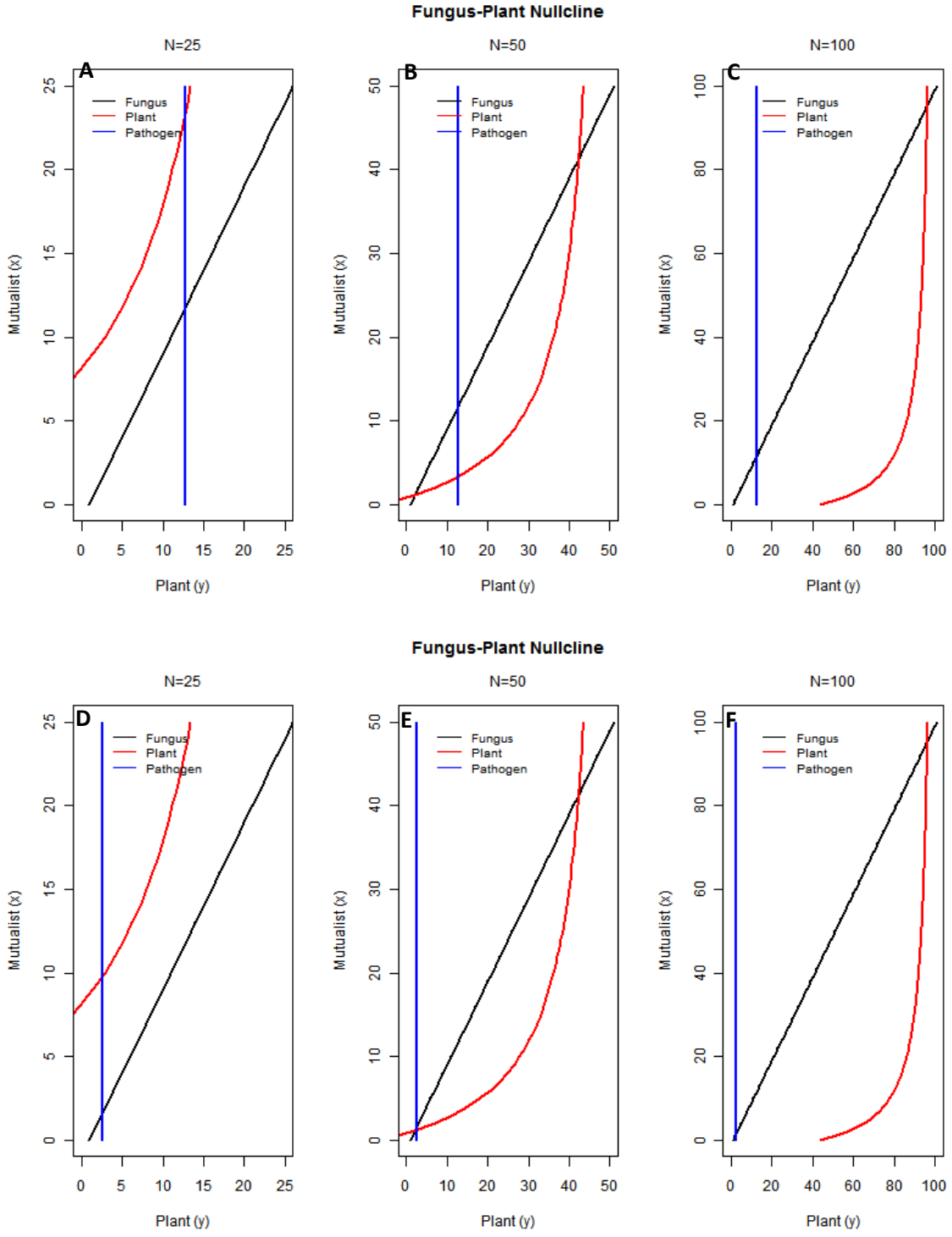


**Figure 5.5. Numerical integration across the plant-mutualist nullcline space, constant c=1.75.** Numerical integration across the plant-mutualist nullcline space under varying nutrient inputs at a constant enemy effective rate. These dynamics inform the nullcline dynamics demonstrated in Figure 5.4A,C. Nutrient rates are N=25 (obligate mutualist, panel A) and N=100 (facultative mutualist, panel B). In order to ensure equilibrium dynamics, a 300 step time transient was run and the following 300 steps are graphed below.

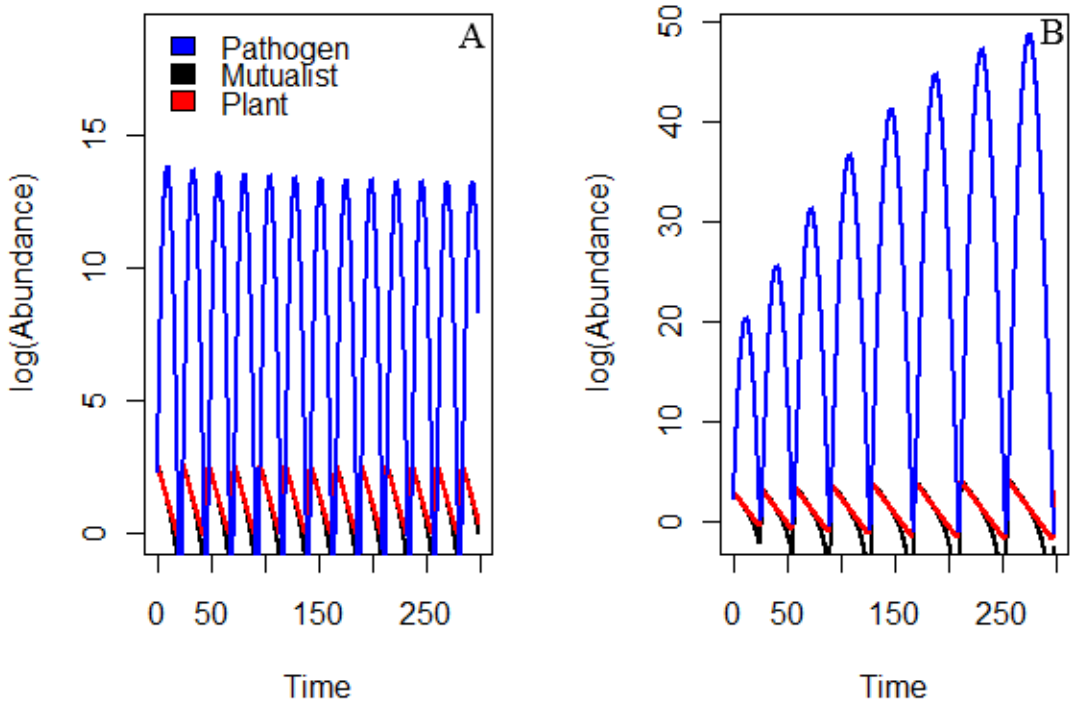


**Table 5.1a. Nondimensionalized parameters and their dimensionalized components, as well as their biological meaning.**

| NonDimensionalized Parameter | Components | Biological Meaning |
|---|---|---|
| f | $\frac{\bar{d}}{\overline{df}}$ | Scaled plant death rate |
| i | $\frac{\bar{\imath}}{\overline{df\bar{\jmath}}}$ | Scaled resource uptake of plant |
| k | $\frac{\bar{k}}{\bar{\jmath}}$ | Scaled benefit of mutualism to plant |
| n | $\overline{nTOT}\frac{\bar{r}*\bar{\jmath}}{\overline{df}}$ | Scaled productivity |
| d | $\frac{\overline{dw}}{\overline{df}}$ | Scaled enemy loss |
| c | $\frac{\bar{c}}{\overline{(\bar{r}*\bar{\jmath})}}$ | Scaled enemy benefit from plant |
| b | $\frac{\bar{b}*\overline{df}}{\overline{(\bar{r}*\bar{\jmath})}}$ | Plant self-limitation in pathogen benefit |

**Table 5.1b. Dimensionalized parameters and their biological meaning and source.**

| Dimensionalized Parameter | Units | Meaning | Value | Source |
|---|---|---|---|---|
| $\overline{nTOT}$ | g/m$^2$ | Maximum soil nutrient concentration | Varies | |
| $\overline{df}$ | nmol/m$^2$/day | Mutulist biomass loss rate (estimated by respiration rate) | 0.0211 | (Staddon et al. 2003) |
| $\bar{d}$ | nmol/m$^2$/day | Plant biomass loss rate (estimated by respiration rate) | 0.06829 | (Fredeen and Field 1995) |
| $\bar{\imath}$ | g/day/ m$^2$ | Resource uptake rate of plant in absence of mutualist | 0.001065 | Experiment |
| $\bar{c}$ | m$^2$/g/s | Viral growth per gram of plant | 0.1* | Estimated |
| $\bar{r}$ | m$^2$/g/s | Rate of resource gained by the mutualist from the plant | 4.323651 | Experiment |
| $\bar{k}$ | m$^2$/g | Plant nutrient uptake benefit from mutualist | 0.154* | (Newman and Ritz 1986) |
| $\overline{dw}$ | | Virus death rate | 0.074 | (Eweida et al. 1988) |
| $\bar{J}$ | g/m$^2$ | Mutualist self-limitation in benefit from plant | 0.0218 | (Jakobsen and Rosendahl 1990) |
| $\bar{l}$ | m$^2$/g | Pathogen interference with nutrient uptake | NA | |
| $\bar{b}$ | m$^2$/g | Plant self-limitation in benefit to enemy | NA | |

*Unless varied for parameter exploration

# CHAPTER VI: CONCLUSIONS

It has long been acknowledged that plant species interact with biotic and abiotic factors, but such research has tended to focus on simple pairwise biotic interactions (Piculell et al. 2008, Tylianakis et al. 2008). The progression from two species interactions to multispecies interactions is an area of developing interest in ecological research (Bruno et al. 2003, Gehring and Bennett 2009, Bennett 2010). My thesis examines the interaction of two classes of microorganisms (mutualists and pathogens) as they relate to one another and their shared host in the context of changing abiotic conditions. We found that 1) mutualists alter pathogen dynamics but mutualistic strategy determines the direction of change, 2) pathogens alter mutualist dynamics and 3) abiotic context can change the relationship between host, mutualists and pathogens.

The identity of the commodities exchanged in a host-mutualist relationship may determine the effects of the mutualism on a pathogen infecting the host (Bronstein and Barbosa 2002, Ferriere et al. 2002). My thesis suggests that mutualistic strategy is important for determining the direction of change by which mutualists alter pathogen dynamics (Chapter 2, 3). In a greenhouse experiment, the protection mutualist foliar endophytic fungi decreased relative viral titer (Chapter 3). This may have been due to decreased vector feeding duration (Power and Gray 1995) as a result of endophyte-induced alkaloid production that deters arthropod feeding (Clay and Schardl 2002). However, this did not translate into decreased pathogen prevalence for endophyte-

infected plants in the field (Chapter 4). In contrast, by altering host nutrition, resource mutualists may stimulate enemy populations (Bennett et al. 2006). Mycorrhizal fungi are a type of resource mutualist characterized by the exchange of carbohydrates from to the plant for less mobile mineral nutrients, particularly phosphorus (Smith and Read 2008); this exchange of carbon for phosphorous may be important for viral pathogens which can be phosphorus limited if the host has a high C:P ratio (Clasen and Elser 2007). In a greenhouse experiment, increased leaf phosphorus concentration did not correspond to increased relative virus titer, but when carbon was abundant (under elevated $CO_2$), the mutualistic association increased relative viral titer (Chapter 2).

Second, both mathematical theory and empirical work indicated that a pathogen can alter host-mutualist dynamics (Chapters 2, 5), which implies that a pathogen may impact long-term co-existence of a host and mutualist (Chapter 5). Under elevated $CO_2$, virus infection stimulated mycorrhizal colonization (Chapter 2). While the mechanism for this result remains unclear, it is possible that an increase in accessibility to plant carbon as a result of phloem degeneration caused by the virus (Irwin and Thresh 1990, D'Arcy 1995) leads to more available carbon for the fungus and thus stimulates colonization. These results suggest the possibility that the virus derives a fitness benefit by stimulating its host to invest more in a mutualism. Furthermore, we were able to use a dynamic systems model to demonstrate that the introduction of an enemy can disrupt the stability of a mutualist-host relationship by inducing cyclic dynamics and imposing a threshold in nutrient supply necessary for co-existence (Chapter 5).

Finally, changing abiotic environmental conditions can modify the relationships between host, mutualists and pathogens. It has previously been hypothesized that the

impact of global change on plant communities may be mediated through indirect effects, including via pathogens (Burdon et al. 2006). We tested this hypothesis with both arbuscular mycorrhizal fungi and foliar endophytic fungi. While experimentally elevated $CO_2$ spurred a pathogen and mutualist to reciprocally increase each other's abundances, this did not translate to significant changes in host biomass (Chapter 2). On the other hand, experimentally elevated precipitation and temperature altered virus prevalence in the field (Chapter 4). Specifically, in the final year of a three year experiment, increased precipitation increased viral prevalence for endophyte-infected plants (Chapter 4). Furthermore, increased temperature increased virus prevalence, but this affect does not appear to be driven by vector behavior as vector abundance was higher for plots with increased heat compared to those with ambient temperature (Chapter 4). As different viral species are transmitted by different vector species (Power and Gray 1995), it is possible that different vectors will have different responses to increased temperature and / or precipitation, which will lead to variation in viral species response to these abiotic vectors. Our results supported this hypothesis, with some virus species increasing in prevalence, and others decreasing in prevalence under elevated precipitation and temperature (Chapter 4). Furthermore, these differential responses among virus species have important implications for co-infection dynamics (Chapter 4).

Overall, my thesis demonstrates that mutualists and pathogens can have important impacts on not only the host but also on the success of each other. Such dynamics can be further modified by changes in the abiotic environment. Further study of the combined effects of abiotic and microbial drivers in such a broader ecological context may be key to understanding and predicting large-scale changes to ecosystems (Treseder 2004,

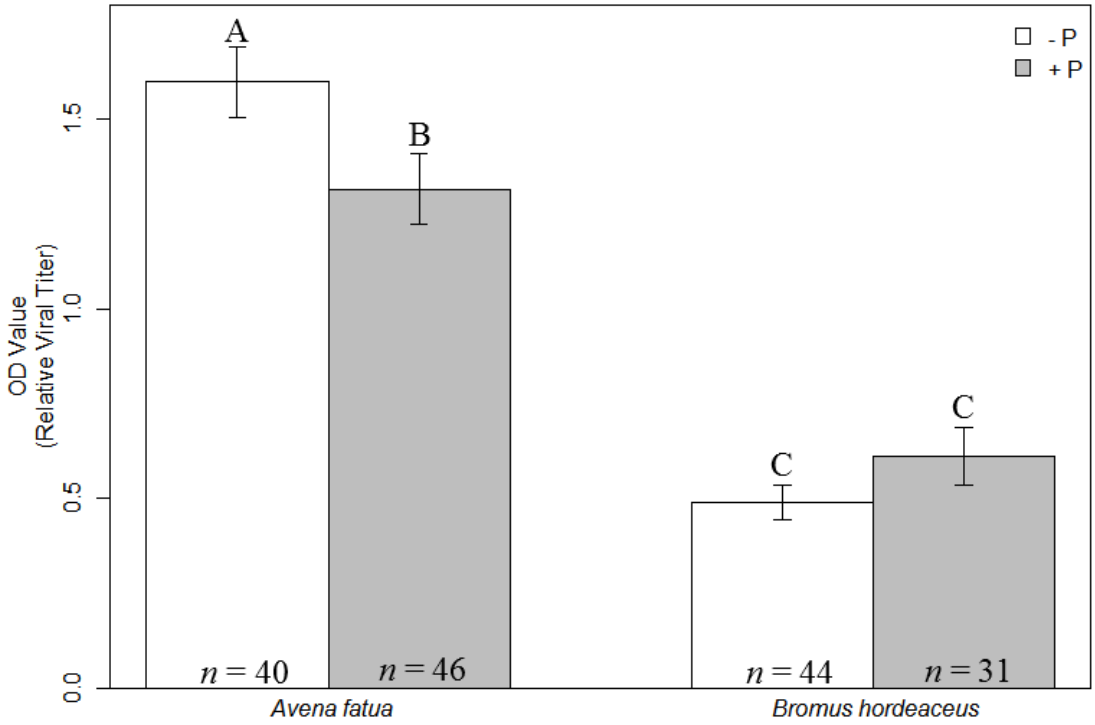
Suding et al. 2008), particularly as they relate to climate change. Further, the impact of global change on plant communities may be mediated through indirect effects, including via pathogens (Burdon et al. 2006). Precipitation, temperature, and atmospheric $CO_2$ are all expected to continue to increase for the foreseeable future (IPCC 2001). Thus, in order to make accurate projections about ecosystem, community or population dynamics, changes in microorganisms must be included in those projections.

# APPENDICES

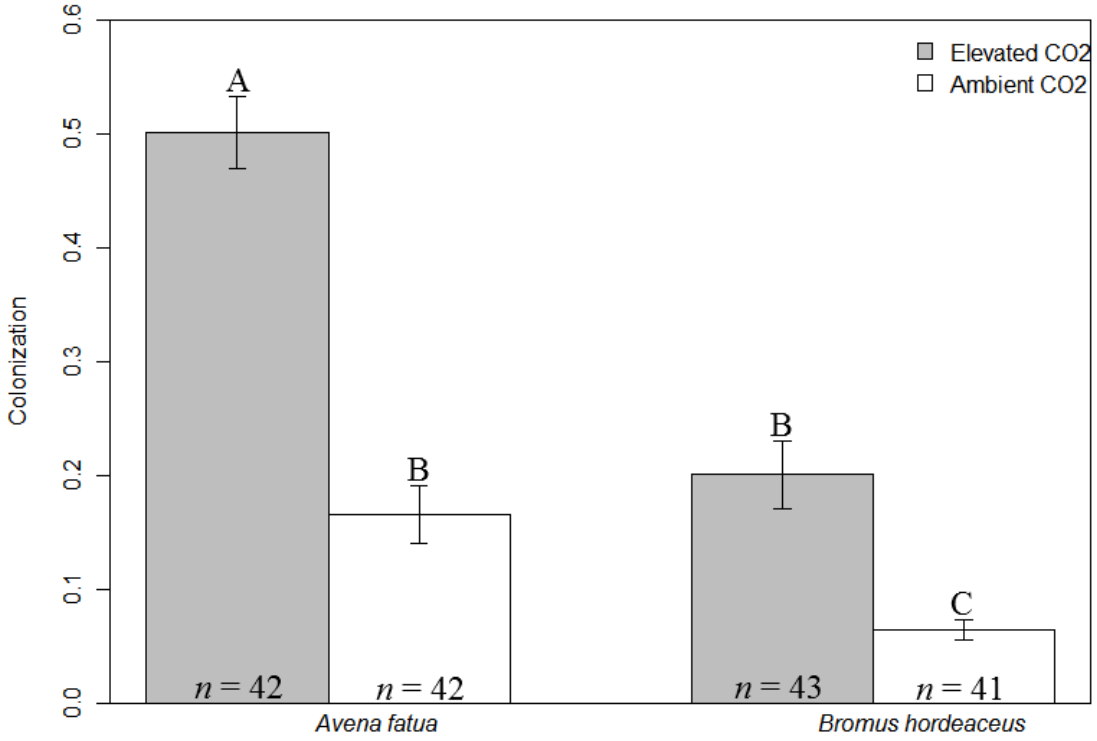
## Appendix A Supplementary Material for Chapter 2.

### A1. Supplementary Figures

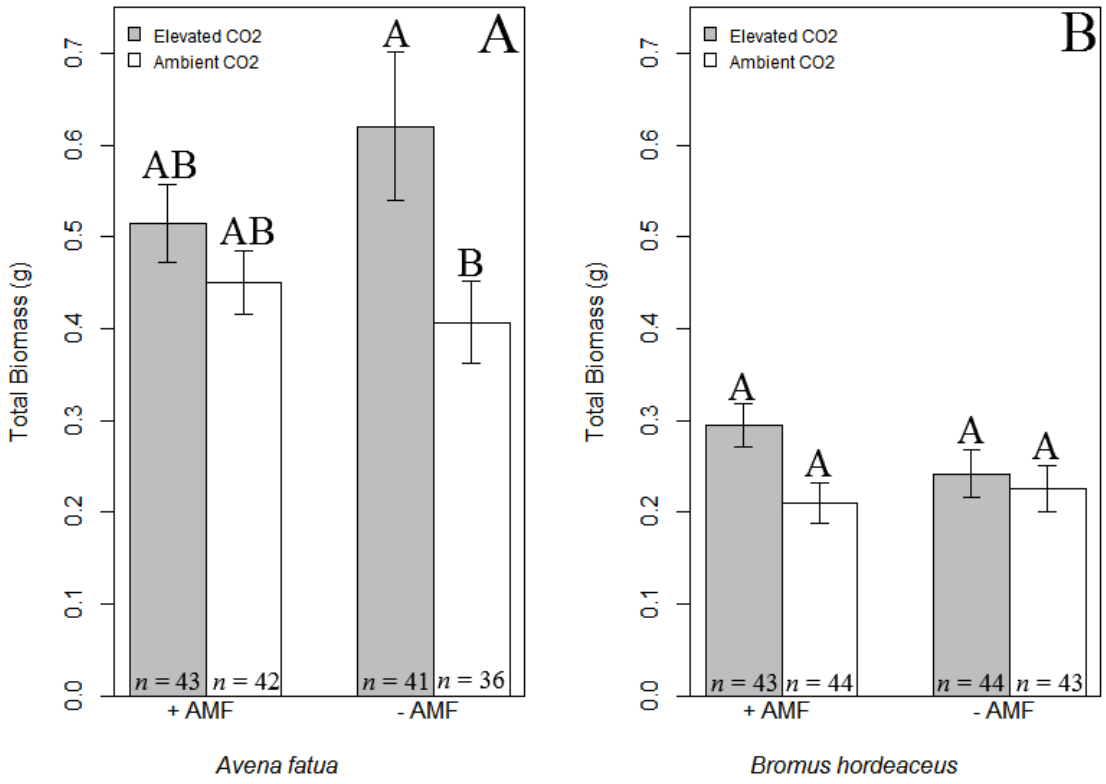
**A1.1. The role of phosphorus addition in altering viral titer.** Across mycorrhizal status, phosphorus addition (+P vs. –P) decreased relative viral titer as measured by ELISA Optical Density (OD) value for (A) *A. fatua* but not for (B) *B. hordeaceus*. Data shown are means ± SEM; letters indicate significant pairwise differences between means (Tukey's HSD; $p<0.05$).



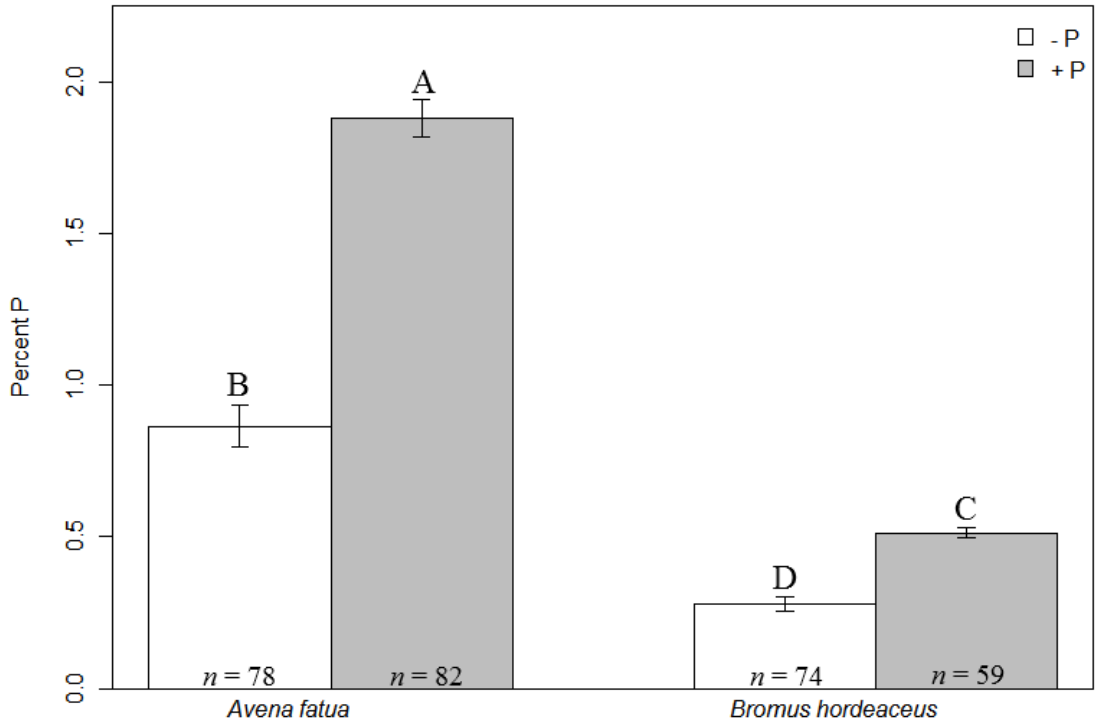
**A1.2. Role of elevated CO2 and host species on root colonization by AMF.** Elevated $CO_2$ increased root colonization for both *Avena fatua* and *Bromus hordeaceus*. Data shown are means ± SEM; letters indicate significant pairwise differences between means (Tukey's HSD; $p<0.05$).



**A1.3.Effect of CO2 and host species on total biomass.** Elevated $CO_2$ increased total plant biomass of (A) non-mycorrhizal (–AMF) *Avena fatua*, but not mycorrhizal (+AMF) *A. fatua*, or (B) *Bromus hordeaceus*. Data shown are means ± SEM; letters indicate significant pairwise differences between means within each figure panel (Tukey's HSD; $p<0.05$).



**A1.4. Role of phosphorus and host species in altering leaf phosphorus concentration.** Across mycorrhizal status, adding phosphorus (+P vs. –P) increased leaf phosphorus concentration (Percent P) for both species, and more for *A. fatua* than *B. hordeaceus*. Data shown are means ± SEM; letters indicate significant pairwise differences between means (Tukey's HSD; $p<0.05$).



## A2. Full Statistical Models.

The following tables present the full statistical models. The following statistical models assess the effects of the four experimental factors: elevated concentration of atmospheric $CO_2$, infection with *Barley yellow dwarf virus – PAV* (BYDV), phosphorus fertilization (P), association with arbuscular mycorrhizal fungi (AMF), and plant species identity (Plant.Species). For analysis details, see the *Methods* section. (Significance codes for all tables: $P < 0.001$ '***'; $0.001 < P < 0.01$ '**'; $0.01 < P < 0.05$ '*'; $0.05 < P < 0.1$ '.')

***Table A2.1. Model of Optical Density values, estimating relative virus titer.***
*Analyses for only virus-infected plants.*

| | numDF | denDF | F-value | p-value | |
|---|---|---|---|---|---|
| (Intercept) | 1 | 141 | 672.9046 | <.0001 | *** |
| CO2 | 1 | 4 | 2.0610 | 0.2244 | |
| AMF | 1 | 141 | 5.8797 | 0.0166 | * |
| P | 1 | 141 | 0.2103 | 0.6472 | |
| Plant.Species | 1 | 141 | 127.4004 | <.0001 | *** |
| CO2:AMF | 1 | 141 | 4.6221 | 0.0333 | * |
| CO2:P | 1 | 141 | 1.4982 | 0.2230 | |
| AMF:P | 1 | 141 | 1.9809 | 0.1615 | |
| CO2:Plant.Species | 1 | 141 | 0.5847 | 0.4458 | |
| AMF:Plant.Species | 1 | 141 | 0.4695 | 0.4944 | |
| P:Plant.Species | 1 | 141 | 4.2592 | 0.0409 | * |
| CO2:AMF:P | 1 | 141 | 0.7243 | 0.3962 | |
| CO2:AMF:Plant.Species | 1 | 141 | 0.0005 | 0.9819 | |
| CO2:P:Plant.Species | 1 | 141 | 0.5054 | 0.4783 | |
| AMF:P:Plant.Species | 1 | 141 | 2.5531 | 0.1123 | |
| CO2:AMF:P:Plant.Species | 1 | 141 | 0.0536 | 0.8173 | |

***Table A2.2. Model of percent hyphal colonization.***
*Analyses for only +AMF plants.*

| | numDF | denDF | F-value | p-value | |
|---|---|---|---|---|---|
| (Intercept) | 1 | 148 | 389.2756 | <.0001 | *** |
| CO2 | 1 | 4 | 97.1052 | 0.0006 | ** |
| BYDV | 1 | 148 | 12.6762 | 0.0005 | ** |
| P | 1 | 148 | 8.6521 | 0.0038 | ** |
| Plant.Species | 1 | 148 | 65.8728 | <.0001 | *** |
| CO2:BYDV | 1 | 148 | 11.3842 | 0.0009 | ** |
| CO2:P | 1 | 148 | 10.4575 | 0.0015 | ** |
| BYDV:P | 1 | 148 | 0.1737 | 0.6774 | |
| CO2:Plant.Species | 1 | 148 | 13.6390 | 0.0003 | ** |
| BYDV:Plant.Species | 1 | 148 | 1.0928 | 0.2976 | |
| P:Plant.Species | 1 | 148 | 1.5033 | 0.2221 | |
| CO2:BYDV:P | 1 | 148 | 1.3709 | 0.2435 | |
| CO2:BYDV:Plant.Species | 1 | 148 | 0.0004 | 0.9834 | |
| CO2:P:Plant.Species | 1 | 148 | 1.0642 | 0.3040 | |
| BYDV:P:Plant.Species | 1 | 148 | 4.6216 | 0.0332 | * |
| CO2:BYDV:P:Plant.Species | 1 | 148 | 0.0949 | 0.7585 | |

***Table A2.3. Model of Total Plant Biomass.***

| | numDF | denDF | F-value | p-value | |
|---|---|---|---|---|---|
| (Intercept) | 1 | 300 | 839.3783 | <.0001 | *** |
| CO2 | 1 | 4 | 9.8449 | 0.0349 | * |
| BYDV | 1 | 300 | 2.9217 | 0.0884 | . |
| AMF | 1 | 300 | 2.7306 | 0.0995 | . |
| P | 1 | 300 | 0.8499 | 0.3573 | |
| Plant.Species | 1 | 300 | 79.1084 | <.0001 | *** |
| CO2:BYDV | 1 | 300 | 2.1262 | 0.1458 | |
| CO2:AMF | 1 | 300 | 0.3004 | 0.5841 | |
| BYDV:AMF | 1 | 300 | 0.7018 | 0.4028 | |
| CO2:P | 1 | 300 | 0.2365 | 0.6271 | |
| BYDV:P | 1 | 300 | 0.0226 | 0.8805 | |
| AMF:P | 1 | 300 | 0.0019 | 0.9648 | |
| CO2:Plant.Species | 1 | 300 | 0.0886 | 0.7662 | |
| BYDV:Plant.Species | 1 | 300 | 0.0326 | 0.8569 | |
| AMF:Plant.Species | 1 | 300 | 0.0756 | 0.7835 | |
| P:Plant.Species | 1 | 300 | 0.8353 | 0.3615 | |
| CO2:BYDV:AMF | 1 | 300 | 0.0938 | 0.7597 | |
| CO2:BYDV:P | 1 | 300 | 0.0272 | 0.8692 | |
| CO2:AMF:P | 1 | 300 | 0.1678 | 0.6824 | |
| BYDV:AMF:P | 1 | 300 | 0.1709 | 0.6796 | |
| CO2:BYDV:Plant.Species | 1 | 300 | 0.1644 | 0.6854 | |
| CO2:AMF:Plant.Species | 1 | 300 | 4.3878 | 0.0370 | * |
| BYDV:AMF:Plant.Species | 1 | 300 | 2.5077 | 0.1143 | |
| CO2:P:Plant.Species | 1 | 300 | 0.3930 | 0.5312 | |
| BYDV:P:Plant.Species | 1 | 300 | 0.0028 | 0.9581 | |
| AMF:P:Plant.Species | 1 | 300 | 0.0574 | 0.8108 | |
| CO2:BYDV:AMF:P | 1 | 300 | 2.5409 | 0.1120 | |
| CO2:BYDV:AMF:Plant.Species | 1 | 300 | 0.0002 | 0.9884 | |
| CO2:BYDV:P:Plant.Species | 1 | 300 | 0.0162 | 0.8988 | |
| CO2:AMF:P:Plant.Species | 1 | 300 | 1.4087 | 0.2362 | |
| BYDV:AMF:P:Plant.Species | 1 | 300 | 0.0943 | 0.7589 | |
| CO2:BYDV:AMF:P:Plant.Species | 1 | 300 | 1.5617 | 0.2124 | |

***Table A2.4. Model of Root Fraction.***

| | numDF | denDF | F-value | p-value | |
|---|---|---|---|---|---|
| (Intercept) | 1 | 300 | 3406.312 | <.0001 | *** |
| CO2 | 1 | 4 | 3.334 | 0.1419 | |
| BYDV | 1 | 300 | 45.136 | <.0001 | *** |
| AMF | 1 | 300 | 2.005 | 0.1578 | |
| P | 1 | 300 | 2.069 | 0.1513 | |
| Plant.Species | 1 | 300 | 126.211 | <.0001 | *** |
| CO2:BYDV | 1 | 300 | 0.009 | 0.9251 | |
| CO2:AMF | 1 | 300 | 0.166 | 0.6841 | |
| BYDV:AMF | 1 | 300 | 0.039 | 0.8431 | |
| CO2:P | 1 | 300 | 0.701 | 0.4031 | |
| BYDV:P | 1 | 300 | 0.533 | 0.4661 | |
| AMF:P | 1 | 300 | 0.804 | 0.3706 | |
| CO2:Plant.Species | 1 | 300 | 0.095 | 0.7578 | |
| BYDV:Plant.Species | 1 | 300 | 2.468 | 0.1173 | |
| AMF:Plant.Species | 1 | 300 | 1.366 | 0.2435 | |
| P:Plant.Species | 1 | 300 | 1.072 | 0.3014 | |
| CO2:BYDV:AMF | 1 | 300 | 0.001 | 0.9742 | |
| CO2:BYDV:P | 1 | 300 | 1.569 | 0.2113 | |
| CO2:AMF:P | 1 | 300 | 0.132 | 0.7169 | |
| BYDV:AMF:P | 1 | 300 | 1.633 | 0.2023 | |
| CO2:BYDV:Plant.Species | 1 | 300 | 0.160 | 0.6893 | |
| CO2:AMF:Plant.Species | 1 | 300 | 0.002 | 0.9686 | |
| BYDV:AMF:Plant.Species | 1 | 300 | 0.014 | 0.9073 | |
| CO2:P:Plant.Species | 1 | 300 | 0.222 | 0.6377 | |
| BYDV:P:Plant.Species | 1 | 300 | 2.598 | 0.1080 | |
| AMF:P:Plant.Species | 1 | 300 | 1.545 | 0.2148 | |
| CO2:BYDV:AMF:P | 1 | 300 | 1.740 | 0.1881 | |
| CO2:BYDV:AMF:Plant.Species | 1 | 300 | 0.317 | 0.5736 | |
| CO2:BYDV:P:Plant.Species | 1 | 300 | 0.745 | 0.3887 | |
| CO2:AMF:P:Plant.Species | 1 | 300 | 0.882 | 0.3484 | |
| BYDV:AMF:P:Plant.Species | 1 | 300 | 0.433 | 0.5108 | |
| CO2:BYDV:AMF:P:Plant.Species | 1 | 300 | 0.793 | 0.3739 | |

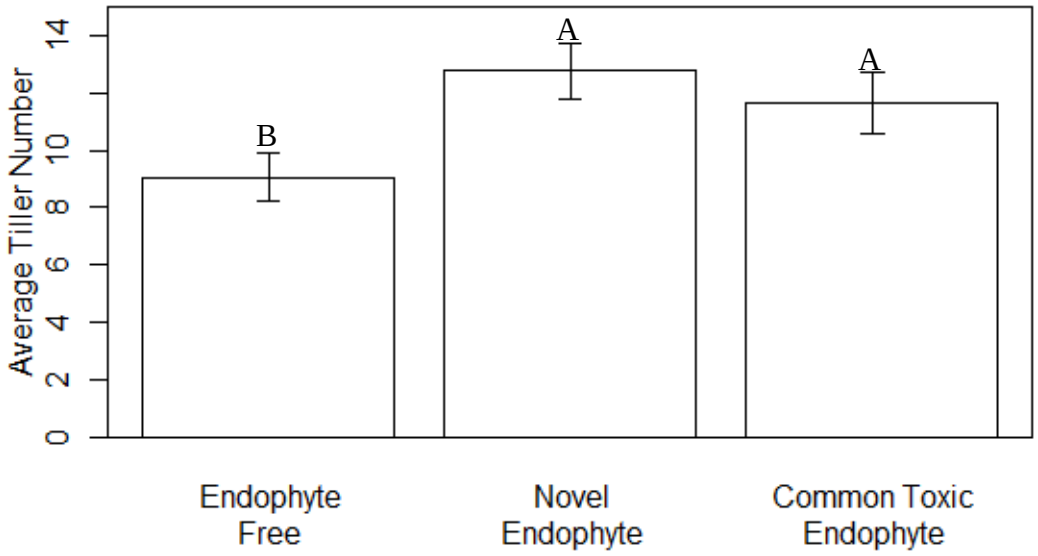
***Table A2.5. Model of Percent Leaf Tissue Phosphorus***

| | numDF | denDF | F-value | p-value | |
|---|---|---|---|---|---|
| (Intercept) | 1 | 257 | 430.0927 | <.0001 | *** |
| CO2 | 1 | 4 | 0.1134 | 0.7533 | |
| BYDV | 1 | 257 | 0.4281 | 0.5135 | |
| AMF | 1 | 257 | 0.2145 | 0.6436 | |
| P | 1 | 257 | 147.2944 | <.0001 | *** |
| Plant.Species | 1 | 257 | 387.8527 | <.0001 | *** |
| CO2:BYDV | 1 | 257 | 1.5980 | 0.2073 | |
| CO2:AMF | 1 | 257 | 0.0016 | 0.9681 | |
| BYDV:AMF | 1 | 257 | 0.0002 | 0.9876 | |
| CO2:P | 1 | 257 | 1.0135 | 0.3150 | |
| BYDV:P | 1 | 257 | 0.2781 | 0.5984 | |
| AMF:P | 1 | 257 | 0.7003 | 0.4035 | |
| CO2:Plant.Species | 1 | 257 | 0.0851 | 0.7708 | |
| BYDV:Plant.Species | 1 | 257 | 5.1518 | 0.0241 | * |
| AMF:Plant.Species | 1 | 257 | 0.0011 | 0.9734 | |
| P:Plant.Species | 1 | 257 | 61.9365 | <.0001 | *** |
| CO2:BYDV:AMF | 1 | 257 | 1.5256 | 0.2179 | |
| CO2:BYDV:P | 1 | 257 | 0.2159 | 0.6426 | |
| CO2:AMF:P | 1 | 257 | 0.0676 | 0.7951 | |
| BYDV:AMF:P | 1 | 257 | 0.8494 | 0.3576 | |
| CO2:BYDV:Plant.Species | 1 | 257 | 1.9750 | 0.1611 | |
| CO2:AMF:Plant.Species | 1 | 257 | 0.1092 | 0.7413 | |
| BYDV:AMF:Plant.Species | 1 | 257 | 0.1335 | 0.7152 | |
| CO2:P:Plant.Species | 1 | 257 | 0.0617 | 0.8040 | |
| BYDV:P:Plant.Species | 1 | 257 | 0.7651 | 0.3826 | |
| AMF:P:Plant.Species | 1 | 257 | 0.4683 | 0.4944 | |
| CO2:BYDV:AMF:P | 1 | 257 | 1.6479 | 0.2004 | |
| CO2:BYDV:AMF:Plant.Species | 1 | 257 | 0.0112 | 0.9157 | |
| CO2:BYDV:P:Plant.Species | 1 | 257 | 0.0104 | 0.9187 | |
| CO2:AMF:P:Plant.Species | 1 | 257 | 1.7082 | 0.1924 | |
| BYDV:AMF:P:Plant.Species | 1 | 257 | 1.9863 | 0.1599 | |
| CO2:BYDV:AMF:P:Plant.Species | 1 | 257 | 0.6841 | 0.4090 | |

## Appendix B Supplementary Materials for Chapter 3

### B1. Supplementary Figures

**B1.1. Tiller number by endophyte infection status for PDF.** Across virus infection status, infection with the novel endophyte or the common toxic endophyte increased tiller production. Data shown are means ± SEM; letters indicate significant pairwise differences between means (Tukey's HSD; $p<0.05$).



**B2. Full Statistical Models**

Tables B2.1, B2.3, B2.5, B2.7, B2.9, B2.11, B2.12, B2.13, B2.17 present the full statistical models that were used to assess the effects of the three experimental factors: infection with *Barley yellow dwarf virus – PAV* (-BYDV vs. +BYDV), host cultivar (PDF vs. KY 31) and endophyte infection (E- vs. CTE+; excluding the novel endophyte PDF 584). Tables B2.2, B2.4, B2.6, B2.8, B2.10, B2.14, B2.15, B2.16, B2.18b present the models that were used to assess the effects of two experimental factors within the PDF cultivar: virus presence (-BYDV vs. +BYDV) and endophyte genotype (Seed.Type: PDF E- vs. PDF E+ vs. PDF 584). For analysis details, see the *Methods* section. (Significance codes for all tables: $P < 0.001$ '***'; $0.001 < P < 0.01$ '**'; $0.01 < P < 0.05$ '*'; $0.05 < P < 0.1$ '.')

***B2.1. Model of Total Plant Biomass***

| | numDF | denDF | F-value | p-value | |
|---|---|---|---|---|---|
| (Intercept) | 1 | 105 | 108.16962 | <.0001 | *** |
| BYDV | 1 | 105 | 15.64519 | 0.0001 | *** |
| Endophyte | 1 | 105 | 0.07895 | 0.7793 | |
| Cultivar | 1 | 105 | 0.03010 | 0.8626 | |
| BYDV:Endophyte | 1 | 105 | 0.15490 | 0.6947 | |
| BYDV:Cultivar | 1 | 105 | 0.78317 | 0.3782 | |
| Endophyte:Cultivar | 1 | 105 | 5.79355 | 0.0178 | * |
| BYDV:Endophyte:Cultivar | 1 | 105 | 0.02726 | 0.8692 | |

***B2.2. Model of Total Plant Biomass:*** *within the PDF cultivar*

| | numDF | denDF | F-value | p-value | |
|---|---|---|---|---|---|
| (Intercept) | 1 | 79 | 94.81344 | <.0001 | *** |
| BYDV | 1 | 79 | 10.07562 | 0.0021 | *** |
| Seed.Type | 2 | 79 | 1.70956 | 0.1876 | |
| BYDV:Seed.Type | 2 | 79 | 0.57409 | 0.5655 | |

***B2.3. Model of Root Biomass***

| | numDF | denDF | F-value | p-value | |
|---|---|---|---|---|---|
| (Intercept) | 1 | 105 | 157.94545 | <.0001 | *** |
| BYDV | 1 | 105 | 21.10247 | <.0001 | *** |
| Endophyte | 1 | 105 | 0.02609 | 0.8720 | |
| Cultivar | 1 | 105 | 2.41767 | 0.1230 | |
| BYDV:Endophyte | 1 | 105 | 0.00516 | 0.9429 | |
| BYDV:Cultivar | 1 | 105 | 0.00497 | 0.9439 | |
| Endophyte:Cultivar | 1 | 105 | 3.28763 | 0.0727 | . |
| BYDV:Endophyte:Cultivar | 1 | 105 | 0.02955 | 0.8638 | |

***B2.4. Model of Root Biomass:*** *within the PDF cultivar*

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|

```
(Intercept)         1    79 103.49826  <.0001 ***
BYDV                1    79  12.83383  0.0006 **
Seed.Type           2    79   2.72537  0.0717 .
BYDV:Seed.Type      2    79   0.48900  0.6151
```

### *B2.5. Model of Shoot Biomass*

```
                      numDF denDF   F-value p-value
(Intercept)               1   105 108.43510  <.0001 ***
BYDV                      1   105  11.93453  0.0008 **
Cultivar                  1   105   0.09953  0.7530
Endophyte                 1   105   0.08930  0.7657
BYDV:Cultivar             1   105   1.25313  0.2655
BYDV:Endophyte            1   105   0.27055  0.6041
Cultivar:Endophyte        1   105   5.91432  0.0167 *
BYDV:Cultivar:Endophyte   1   105   0.02165  0.8833
```

### *B2.6. Model of Shoot Biomass: within the PDF cultivar*

```
                  numDF denDF   F-value p-value
(Intercept)         1    79 104.30478  <.0001 ***
BYDV                1    79   8.58912  0.0044 **
Seed.Type           2    79   1.36517  0.2613
BYDV:Seed.Type      2    79   0.58524  0.5594
```

### *B2.7. Model of Root Fraction*

```
                      numDF denDF  F-value p-value
(Intercept)               1   105 571.1717  <.0001 ***
BYDV                      1   105   6.9477  0.0097 **
Endophyte                 1   105   0.0769  0.7821
Cultivar                  1   105   1.4787  0.2267
BYDV:Endophyte            1   105   2.8354  0.0952 .
BYDV:Cultivar             1   105   0.8626  0.3551
Endophyte:Cultivar        1   105   1.2749  0.2614
BYDV:Endophyte:Cultivar   1   105   0.5225  0.4714
```

### *B2.8. Model of Root Fraction: within the PDF cultivar*

```
                  numDF denDF  F-value p-value
(Intercept)         1    79 608.4623  <.0001 ***
BYDV                1    79   6.8896  0.0104 *
Seed.Type           2    79   0.2902  0.7489
BYDV:Seed.Type      2    79   2.0979  0.1295
```

### *B2.9. Model of Tiller Number*

```
                         Estimate Std. Error z value Pr(>|z|)
(Intercept)               2.40214    0.08337  28.813  <.0001 ***
Cultivar                 -0.11783    0.10303  -1.144  0.2528
Endophyte                 0.03526    0.10486   0.336  0.7367
BYDV                     -0.02720    0.11508  -0.236  0.8132
Cultivar:Endophyte        0.17490    0.14938   1.171  0.2416
Cultivar:BYDV            -0.18578    0.17017  -1.092  0.2749
Endophyte:BYDV           -0.44573    0.17173  -2.595  0.0095 **
Cultivar:Endophyte:BYDV   0.55688    0.23906   2.329  0.0198 *
```

### *B2.10. Model of Tiller Number: within the PDF cultivar*

```
                       Estimate Std. Error z value Pr(>|z|)
(Intercept)             2.28447    0.09304  24.553  < 2e-16 ***
BYDV                   -0.21641    0.12562  -1.723 0.0849 .
Seed.TypePDF584         0.37589    0.10263   3.663 0.0003 ***
Seed.TypePDFE+          0.20203    0.10636   1.900 0.0575 .
BYDV:Seed.TypePDF584   -0.04087    0.16249  -0.252 0.8014
```

```
BYDV:Seed.TypePDFE+     0.12663    0.16642   0.761 0.4468
```

### *B2.11. Model of nymph production by the aphid* Rhopalosiphum padi

```
                       Estimate Std. Error z value Pr(>|z|)
(Intercept)              1.0531     0.1525   6.906  <.0001 ***
Cultivar                -0.6702     0.2621  -2.557  0.0106 *
BYDV                    -0.8168     0.2755  -2.965  0.0030 **
Endophyte               -0.4733     0.2515  -1.882  0.0598 .
Cultivar:BYDV            0.4983     0.4288   1.162  0.2452
Cultivar:Endophyte       0.4470     0.3984   1.122  0.2618
BYDV:Endophyte          -0.2049     0.4764  -0.430  0.6672
Cultivar:BYDV:Endophyte  0.3490     0.6637   0.526  0.5990
```

### *B2.12. Model of adult aphid abundance for the aphid* Rhopalosiphum padi

```
                       Estimate Std. Error z value  Pr(>|z|)
(Intercept)             0.87547    0.16667   5.253   <.0001 ***
Cultivar               -0.21622    0.24952  -0.867   0.3862
BYDV                    0.34831    0.21768   1.600   0.1096
Endophyte              -0.62415    0.28868  -2.162   0.0306 *
Cultivar:BYDV          -0.41977    0.34483  -1.217   0.2235
Cultivar:Endophyte      0.50391    0.39935   1.262   0.2070
BYDV:Endophyte          0.01942    0.37604   0.052   0.9588
Cultivar:BYDV:Endophyte 0.38851    0.53138   0.731   0.4647
```

### *B2.13 Model of total aphid abundance for the aphid* Rhopalsiphum padi

```
                       Estimate Std. Error z value Pr(>|z|)
(Intercept)             1.85630    0.10206  18.188  <.0001 ***
Cultivar               -0.52130    0.16721  -3.118  0.0018 **
BYDV                   -0.16990    0.15087  -1.126  0.2601
Endophyte              -0.52506    0.17113  -3.068  0.0022 **
Cultivar:BYDV           0.11583    0.24254   0.478  0.6330
Cultivar:Endophyte      0.46303    0.25851   1.791  0.0733 .
BYDV:Endophyte         -0.06272    0.25581  -0.245  0.8063
Cultivar:BYDV:Endophyte 0.24665    0.37134   0.664  0.5066
```

### *B2.14. Model of nymph production by the aphid* Rhopalosiphum padi: *within the PDF cultivar*

```
Coefficients:
                     Estimate Std. Error z value Pr(>|z|)
(Intercept)           0.38299    0.21320   1.796   0.0724 .
BYDV                 -0.31845    0.32856  -0.969   0.3324
Seed.TypePDF584       0.12783    0.29233   0.437   0.6619
Seed.TypePDFE+       -0.02632    0.30896  -0.085   0.9321
BYDV:Seed.TypePDF584  0.23507    0.43753   0.537   0.5911
BYDV:Seed.TypePDFE+   0.14410    0.46207   0.312   0.7551
```

### *B2.15. Model of adult aphid abundance for the aphid* Rhopalosiphum padi: *within the PDF cultivar*

```
                      Estimate Std. Error z value Pr(>|z|)
(Intercept)           0.65925    0.18570   3.550 0.000385 ***
BYDV                 -0.07146    0.26743  -0.267 0.789312
Seed.TypePDF584       0.06669    0.25834   0.258 0.796290
Seed.TypePDFE+       -0.12025    0.27595  -0.436 0.663010
BYDV:Seed.TypePDF584  0.44413    0.35496   1.251 0.210859
BYDV:Seed.TypePDFE+   0.40793    0.37545   1.087 0.277255
```

### *B2.16 Model of total aphid abundance for the aphid* Rhopalsiphum padi: *within the PDF cultivar*

```
Coefficients:
```

```
                     Estimate Std. Error z value Pr(>|z|)
(Intercept)           1.33500    0.13245  10.079   <2e-16 ***
BYDV                 -0.05407    0.18990  -0.285    0.776
Seed.TypePDF584       0.26101    0.17623   1.481    0.139
Seed.TypePDFE+       -0.06204    0.19376  -0.320    0.749
BYDV:Seed.TypePDF584  0.10671    0.24979   0.427    0.669
BYDV:Seed.TypePDFE+   0.18393    0.26918   0.683    0.494
```

***B2.17. Model of Model of Optical Density values, estimating relative virus titer.***
*Analyses for only virus-infected plants.*

```
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)         2.2200     0.2881   7.707  <.0001 ***
Endophyte          -0.5259     0.3849  -1.366  0.1783
Cultivar           -1.0565     0.3914  -2.699  0.0096 **
Endophyte:Cultivar  0.2556     0.5325   0.480  0.6334
```

***B2.18. Model of Model of Optical Density values, estimating relative virus titer.***
*Analyses for only virus-infected plants.*

***a) within the KY cultivar***

```
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  2.2200     0.3162   7.020 3.74e-07 ***
Endophyte   -0.5259     0.4226  -1.244    0.226
```

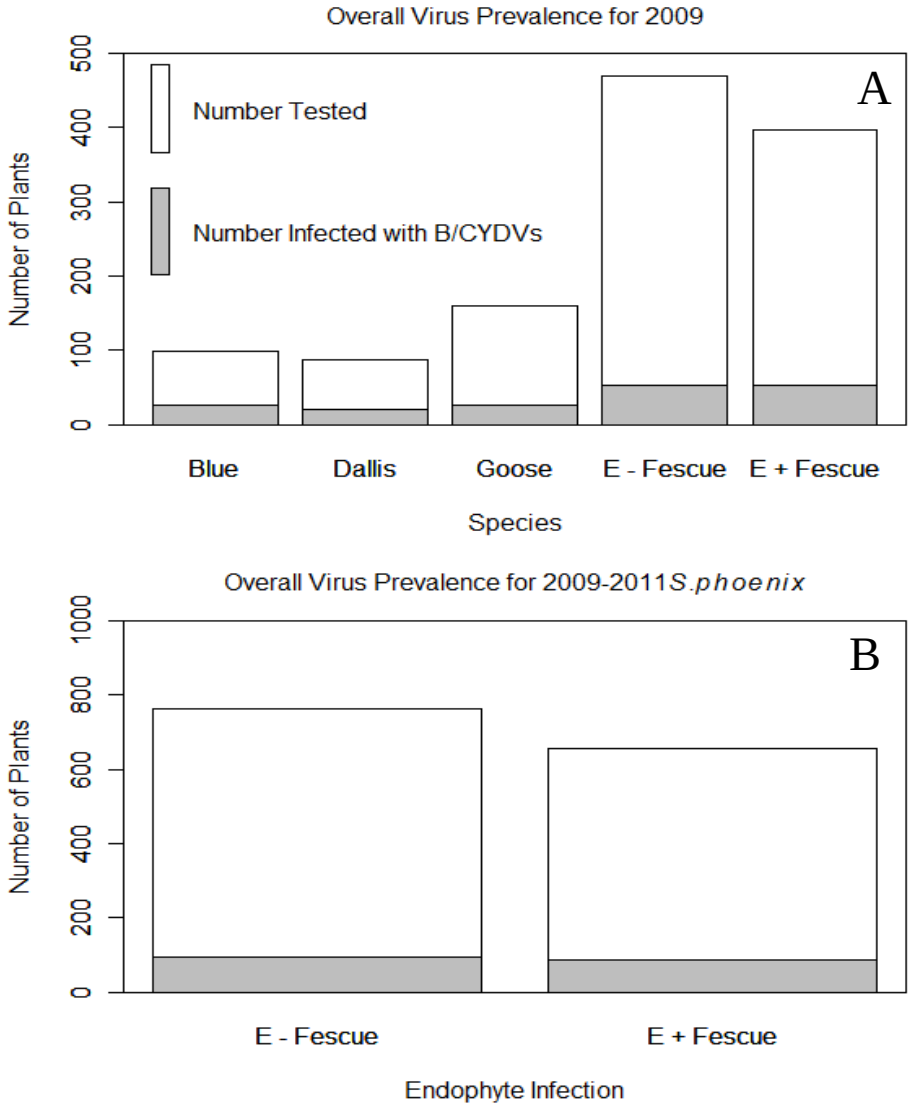
***b) within the PDF cultivar***

```
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)        1.1635     0.2291   5.077 9.81e-06 ***
Seed.TypePDF584   -0.3187     0.3131  -1.018    0.315
Seed.TypePDFE+    -0.2702     0.3182  -0.849    0.401
```

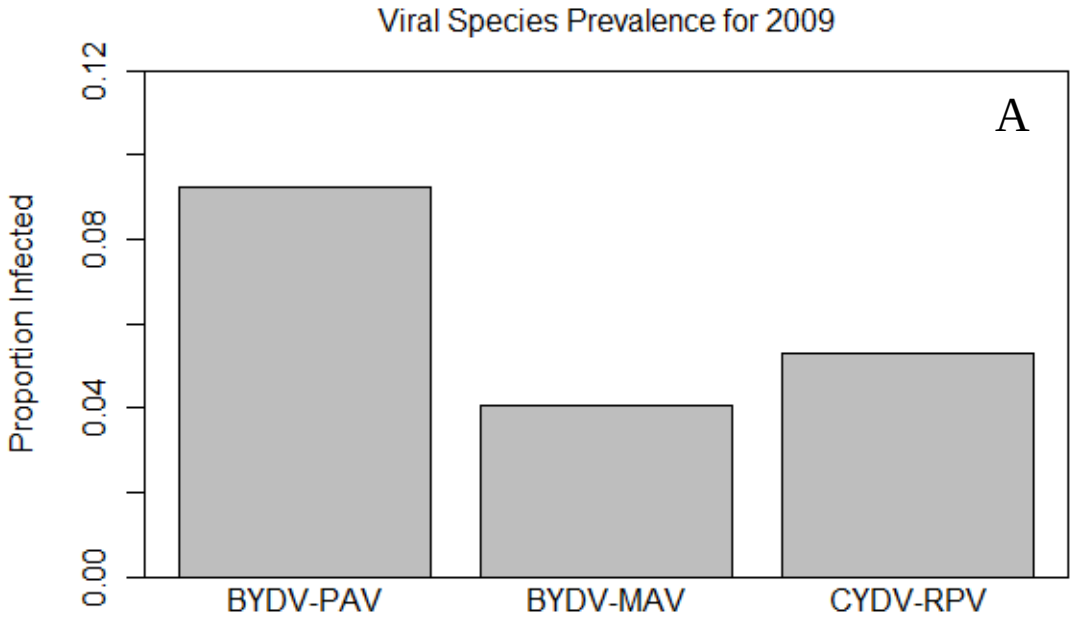
## Appendix C Supplementary Material for Chapter 4

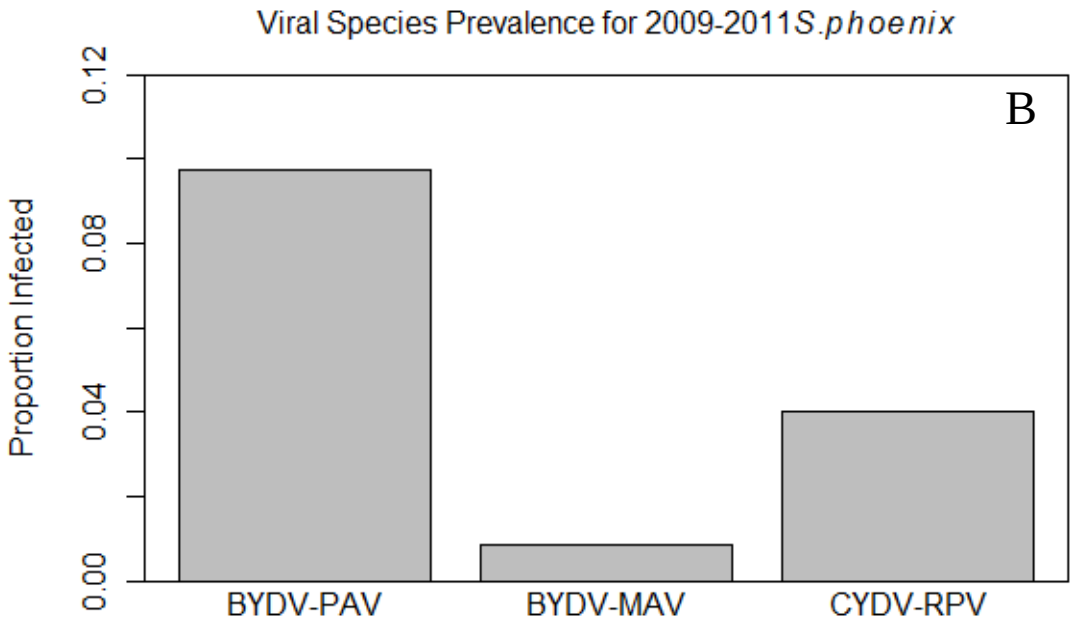
### C1. Supplementary Figures

**C1.1. The proportion of plants infected by a virus varied among host species**. The proportion of plants infected by a virus varied among host species. Total counts of host plants tested are shown in white and infected plants are shown in grey across all experimental units. (A) Virus infection rates for the 2009 data were: 26% for Kentucky bluegrass (26/99), 24% for dallisgrass (21/88), 16% for Indian goosegrass (26/159), 11% for endophyte-free *S. phoenix* ('E- Fescue', 53/470) and 13% for endophyte-infected *S. phoenix* ('E+ Fescue', 53/396). (B) Virus infection rates for the 2009-2011 *S. phoenix* data were: 12.2 % for endophyte-free S. phoenix ('E- Fescue', 93/762) and 13.4% for endophyte-infected *S. phoenix* ('E+ Fescue', 88/657).



**C1.2. The proportion infected with each viral species for all plants.** The proportion infected (number infected / total number tested) for all plants with each viral species (BYDV-PAV, BYDV-MAV, CYDV-RPV) for the 2009 data across host species (A) and the 2009-2011data on *S. phoenix* (B). Plants tested positive for BYDV-PAV most frequently and tested positive for BYDV-MAV least frequently.



## C2. Supplementary Tables

**Table C2.1. Frequencies of climate change treatment × species combinations for aphid counts**. The total number of aphids observed on endophyte-free *Schedonorus phoenix* ('E- Fescue'), endophyte-infected *S. phoenix* ('E+ Fescue') and *Poa pratensis* ('Bluegrass') in each climate change treatment. Heated ('H') plots received an increase in temperature of 3ºC, day and night, year-round. Precipitation ('P') plots received a 30% increase in long-term mean annual precipitation applied during the growing season. Plots designated as 'HP' received both treatments and control ('C') plots were exposed to ambient temperatures and precipitation.

| **Treatment** | **Species** | | | | | |
|---|---|---|---|---|---|---|
| | E- Fescue | | E+ Fescue | | Bluegrass | |
| | Num. Aphids | Num. Plants | Num. Aphids | Num. Plants | Num. Aphids | Num. Plants |
| C | 3 | 36 | 2 | 26 | 9 | 100 |
| H | 11 | 48 | 6 | 30 | 17 | 100 |
| HP | 4 | 48 | 4 | 30 | 12 | 100 |
| P | 4 | 41 | 0 | 24 | 7 | 100 |

**Table C2.2. Model comparisons with DIC values for models describing virus infection in 2009**. Treatments include increased heat ('H'), increased precipitation ('P'), and increased heat x precipitation ('HP'). Heated plots received an increase in temperature of 3ºC, day and night, year-round. Precipitation ('Precip') plots received a 30% increase in long-term mean annual precipitation applied during the growing season. Plots designated as 'Heat x Precip' received both treatments. Species include "blue" (Kentucky Bluegrass, *Poa pratensis*), "dallis" (Dallisgrass, *Paspalum dilatatum)*, "goose" (Goosegrass, *Eleusine indica)* and "pos" (endophyte-infected Tall Fescue *Schedonorus phoenix).*The posterior mean of the deviance minus the deviance of the posterior means (pD) is an approximation of the true number of parameters and is used to calculate the Deviance information criteria (DIC). DIC is an estimate of expected predictive error (lower deviance is better).

| | **Model** | **pD** | **DIC** |
|---|---|---|---|
| **1** | P+ H+ HP + blue + dallis + goose + pos + blueH + dallisH + gooseH + posH + blueP + dallisP + gooseP + posP + blueHP + dallisHP + gooseHP + posHP | 23.1 | 325.4 |
| **2** | P+ H+ HP + blue + dallis + goose + pos + blueH + dallisH + gooseH+ posH + blueP + dallisP + gooseP + posP | 19.3 | 319.3 |
| **3** | P+ H+ HP + blue + dallis + goose + pos + blueH + dallisH + gooseH + posH | 15.0 | 311.1 |
| **4** | P+ H+ HP + blue + dallis + goose + pos + blueP + dallisP + gooseP + posP | 15.2 | 313.6 |
| **5** | P+ H+ HP + blue + dallis + goose + pos | 11.1 | 306.6 |
| **6** | P+ H+ blue + dallis + goose + pos | 9.9 | 304.8 |
| **7** | P+H+HP | 7 | 317.9 |

**Table C2.3. Models of aphid presence compared based on DIC values.** Treatments include increased heat ('H'), increased precipitation ('P'), increased heat and precipitation ('HP'), and endophyte infection ('speciespos'). The posterior mean of the deviance minus the deviance of the posterior means (pD) is an approximation of the true number of parameters and is used to calculate the Deviance information criteria (DIC). DIC is an estimate of expected predictive error (lower deviance is better).

| | Model | pD | DIC |
|---|---|---|---|
| **1** | P + H + speciespos + HP + speciesposP + speciesposH + speciesposHP | 10.3 | 84.4 |
| **2** | P + H + speciespos + HP + speciesposP + speciesposH | 9.8 | 87.3 |
| **3** | P + H + speciespos + HP + speciesposP | 9.1 | 86.2 |
| **4** | P + H + speciespos + HP + speciesposH | 8.9 | 85.3 |
| **5** | P + H + speciespos + HP | 8.3 | 84.5 |
| **6** | P + H + speciespos | 7.2 | 83.2 |
| **7** | P + H + HP | 7.2 | 82.9 |
| **8** | P + H | 6.0 | 81.3 |

## C3. Full Statistical Models

*Frequentist models*. A binomial model was fit with the function 'lmer' of the lme4 package (Bates and Maechler 2009). The random effects variance and standard deviation due to each hexagon was not properly estimated using this method (all estimates are essentially zero) for 2009 community level data (Table C3.1) and aphid abundance data (Table C3.3).

***Table C3.1. Summary for model of virus infection described by heat, precipitation or host species***

```
Generalized linear mixed model fit by the Laplace approximation
Formula: cbind(y, n - y) ~ H * P * species + (1 | hex)
   Data: new.dat1
   AIC   BIC logLik deviance
 150.4 203.6  -54.2    108.4
Random effects:
 Groups Name        Variance   Std.Dev.
 hex    (Intercept) 3.5406e-13 5.9503e-07
Number of obs: 93, groups: hex, 20

Fixed effects:
                           Estimate Std. Error z value Pr(>|z|)
(Intercept)               -1.670684   0.250065  -6.681 2.37e-11 ***
Hhi                       -1.003464   0.442892  -2.266   0.0235 *
Phi                       -0.275226   0.387872  -0.710   0.4780
species2Blue               0.517996   0.530878   0.976   0.3292
species2Dallis             0.518001   0.530878   0.976   0.3292
species2Goose              0.397730   0.495367   0.803   0.4220
species2Pos Fescue         0.048817   0.370646   0.132   0.8952
Hhi:Phi                    0.822970   0.608410   1.353   0.1762
Hhi:species2Blue           1.211688   0.783496   1.547   0.1220
Hhi:species2Dallis         1.175311   0.728035   1.614   0.1064
Hhi:species2Goose          0.330525   0.871755   0.379   0.7046
Hhi:species2Pos Fescue     0.655897   0.604946   1.084   0.2783
Phi:species2Blue           0.540602   0.755927   0.715   0.4745
Phi:species2Dallis        -0.276835   0.980128  -0.282   0.7776
Phi:species2Goose          0.002254   0.653159   0.003   0.9972
Phi:species2Pos Fescue     0.051260   0.549985   0.093   0.9257
Hhi:Phi:species2Blue      -1.296564   1.099479  -1.179   0.2383
Hhi:Phi:species2Dallis    -0.899508   1.579541  -0.569   0.5690
Hhi:Phi:species2Goose     -0.763592   1.182826  -0.646   0.5186
Hhi:Phi:species2Pos Fescue -0.721435   0.854113  -0.845   0.3983
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

***Table C3.2. Summary for model of Tall Fescue* (Schedonorus phoenix) *virus infection described by heat, precipitation, endophyte-infection status or year***

```
Generalized linear mixed model fit by the Laplace approximation
Formula: cbind(y, n - y) ~ H * P * species * year + (1 | hex)
   Data: new.dat2.TF
   AIC   BIC logLik deviance
 160.1 229.8 -55.05    110.1
Random effects:
 Groups Name        Variance Std.Dev.
 hex    (Intercept) 0.065597 0.25612
Number of obs: 120, groups: hex, 20

Fixed effects:
                                    Estimate Std. Error z value Pr(>|z|)
(Intercept)                         -1.69134    0.27673  -6.112 9.85e-10 ***
Hhi                                 -1.01829    0.47485  -2.144   0.0320 *
Phi                                 -0.28291    0.42286  -0.669   0.5035
speciesPos Fescue                    0.04937    0.37434   0.132   0.8951
year2010                             0.17687    0.41886   0.422   0.6728
year2011                            -0.20385    0.80370  -0.254   0.7998
Hhi:Phi                              0.82157    0.65478   1.255   0.2096
Hhi:speciesPos Fescue                0.67877    0.60984   1.113   0.2657
Phi:speciesPos Fescue                0.05415    0.55435   0.098   0.9222
Hhi:year2010                         0.78439    0.66596   1.178   0.2389
Hhi:year2011                         1.88627    1.06057   1.779   0.0753 .
Phi:year2010                        -0.12045    0.64022  -0.188   0.8508
Phi:year2011                       -16.00949 2280.20132  -0.007   0.9944
speciesPos Fescue:year2010          -0.08276    0.61182  -0.135   0.8924
speciesPos Fescue:year2011          -0.88737    1.34242  -0.661   0.5086
Hhi:Phi:speciesPos Fescue           -0.72132    0.86135  -0.837   0.4024
Hhi:Phi:year2010                    -1.17916    1.01997  -1.156   0.2477
Hhi:Phi:year2011                    14.60172 2280.20157   0.006   0.9949
Hhi:speciesPos Fescue:year2010      -1.20523    1.00867  -1.195   0.2321
Hhi:speciesPos Fescue:year2011       0.15921    1.64937   0.097   0.9231
Phi:speciesPos Fescue:year2010      -0.23686    0.93635  -0.253   0.8003
Phi:speciesPos Fescue:year2011      17.07624 2280.20172   0.007   0.9940
Hhi:Phi:speciesPos Fescue:year2010   1.86125    1.49216   1.247   0.2123
Hhi:Phi:speciesPos Fescue:year2011 -15.91917 2280.20219  -0.007   0.9944
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

***Table C3.3. Summary for model of aphid presence described by heat, precipitation, or their interaction***

```
Generalized linear mixed model fit by the Laplace approximation
Formula: cbind(y, n - y) ~ P * H * species + (1 | hex)
   Data: TF
   AIC   BIC logLik deviance
 41.78 56.76 -11.89    23.78
Random effects:
 Groups Name        Variance Std.Dev.
 hex    (Intercept)  0         0
Number of obs: 39, groups: hex, 20

Fixed effects:
                            Estimate Std. Error z value Pr(>|z|)
(Intercept)                 -2.39789    0.60302  -3.976 6.99e-05 ***
Phi                          0.17328    0.80041   0.216   0.8286
Hhi                          1.18487    0.69395   1.707   0.0877 .
speciesPos Fescue           -0.08702    0.95148  -0.091   0.9271
Phi:Hhi                     -1.35815    1.01554  -1.337   0.1811
Phi:speciesPos Fescue      -18.18778 5771.46059  -0.003   0.9975
Hhi:speciesPos Fescue       -0.08625    1.10976  -0.078   0.9380
Phi:Hhi:speciesPos Fescue   18.88711 5771.46067   0.003   0.9974
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

## Appendix D Supplementary Material for Chapter 5

### Dimensionalized Equations

Dimensionalized differential equations describing the changes in abundances of the plant host $\left(\frac{dY}{dt}\right)$, it's mutualist $\left(\frac{dX}{dt}\right)$ and microbial enemy $\left(\frac{dW}{dt}\right)$. Non-dimensionalization was undertaken to simplify the dimensionalized equations. Thus equations (2) were scaled by biomass and time. Doing so folded parameters $\bar{b}$ (pathogen modified for the interaction with plants) and $\bar{l}$ plant modifier for the interaction with pathogens) into larger non-dimensionalized parameters (Table 5.1a,b).

$$\frac{dW}{dt} = Y \cdot \bar{c}\left(\frac{W}{1 + Y \cdot \bar{b}}\right) - \left(\overline{dw} \cdot W\right)$$

$$\frac{dY}{dt} = \bar{\imath} \cdot Y\left(\frac{1 + \bar{k} \cdot X}{1 + \bar{l} \cdot W}\right)(nTOT - Y) - \left(\bar{d} \cdot Y\right)$$

$$\frac{dX}{dt} = \bar{r} \cdot Y\left(\frac{X}{1 + X \cdot \bar{\jmath}}\right) - \overline{df} \cdot X \qquad (2)$$

### Parameterization

Literature values were chosen based on studies that used the same species of host (*Avena fatua*), mycorrhizae (*Glomus* spp.) and/or pathogen (barley and cereal yellow dwarf viruses - BYDV). The value of the *d* parameter (plant respiration rate) was calculated from (Fredeen and Field 1995) in which *A. fatua* monoculture grown in phytocells at ambient $CO_2$. The belowground respiration was reported in units μmol m-2 s-l but converted to nmol m-2 day-1. The fungal respiration rate ($\overline{df}$) was calculated using data from (Staddon et al. 2003) in which carbon turnover in the extraradical mycelium was measured. Fungal respiration rate was

calculated as moles of carbon per hyphal density as a function of root biomass per rate of hyphal turnover and reported in units nmol m-2 day-1. The virus death rate ($\overline{dw}$) was calculated using data from (Eweida et al. 1988) in which the concentration of BYDV virus particles was measured over time. Virus death rate was calculated as the exponential decay ($\lambda = \frac{-\ln\left(\frac{N(t)}{N_0}\right)}{t}$) of virus concentration. To obtain $\overline{dw}$, coefficients were extracted from a linear model fit to the log ratios of virus concentration (the instantaneous lambda). The per fungal biomass increase in resource uptake ($\bar{k}$) is the instantaneous uptake rate of $^{32}$P from the soil reported in (Newman and Ritz 1986). The per plant effect on fungal growth ($\bar{\jmath}$) is evaluated as the density of hyphae divided by the amount of carbon from the plant. This value was obtained from (Jakobsen and Rosendahl 1990) in units (mg C dm -2 h -1) and converted to g C m-2 by adjusting the rate to account for the amount of time the experiment was run and converting mg C to g C.

Two parameters were calculated from a greenhouse experiment. The resource uptake rate of the plant in absence of a mutualist ($\bar{\imath}$) was evaluated as the maximum growth rate of the plant. This value was obtained by using growth values over time and logistics. Specifically, we evaluated the log of the ratio of the longest leaf lengths of plants without mycorrhizae at the end of the experiment to the longest leaf lengths at the beginning of the experiment by the longest leaf length at the beginning of the experiment. The slope of this line represents the instantaneous rate of growth or the maximum growth rate of the plant ($\bar{\imath}$). Resource gained as a function of plant density ($\bar{r}$) was also calculated from the same greenhouse experiment. This value was calculated by multiplying the host photosynthetic capacity by its leaf area ratio for plants infected with virus and associating with mycorrhizae.

**Greenhouse experimental design and treatments**

One pathogen system which has been explored more fully is the influence of Barley and Cereal Yellow Dwarf Viruses (B/CYDVs) on the conversion of pacific grasslands from native perennials to invasive annuals (Malmstrom et al. 2005b, Borer et al. 2007). B/CYDVs are a group of aphid-vectored generalist viral pathogens that infect over 150 crop and noncrop grasses (D'Arcy 1995, Halbert and Voegtlin 1995). Infection is systemic and localized to the phloem where it causes necrosis and disruption of carbohydrate translocation (Irwin and Thresh 1990, D'Arcy 1995). This induces many physiological host responses including: stunted plant growth (Malmstrom et al. 2005a) , reduced root/shoot ratio (Kolb et al. 1991) and reduced longevity.

In January 2008 a five month experiment was conducted factorially manipulating arbuscular mycorrhizal fungi (mycorrhizal and no-mycorrhizal) and virus (infected and uninfected). For this experiment we used the Eurasian annual host plant *Avena fatua.* This host plant was chosen because it is colonized by mycorrhizal fungi (Hu et al. 2005, Rillig 2006) and is a hosts for B/CYDVs (Malmstrom et al. 2005b). Experimental seed was hand-collected in Oregon.

Individual plants were grown in D60 Deeppots (Steuwe and Sons Inc., Oregon, USA). Each plant received 600 g of steam sterilized soil in a mixture of one part soil (Metromix 400) with two parts of pure sand (by mass). To inoculate plants with mycorrhizal fungi, we added 50 g of active mycorrhizal spore inoculum per pot. We used inoculum which consists of spores from the mycorrhizal fungal species *Glomus intraradices, Gigaspora margarita,* and *Scutellospora heterogama*. To control for potential changes in nutrient content due to the inoculum, control plants received 50 g of autoclave sterilized inoculum. All pots received 50 mL of microbial

filtrate solution filtrated by Whatman No. 1 filter paper from 10.0 g AM inoculum (in which mycorrhizal spores were removed) to correct for possible differences in the microbial community and mineral content between mycorrhizal and no mycorrhizal treatments.

To infect plants with virus we used an isolate of *Barley yellow dwarf virus – PAV* obtained from a naturally infected *Bromus vulgaris* and maintained in *Avena sativa* cultivar Coast Black Oats. Virus inoculations occurred approximately two weeks after germination. Uninfected aphids of the species *Rhopalosiphum padi* (L.) were fed in petri dishes for 72 hours on infected plant tissue. Five infected aphids were then transferred to each experimental plant, at which time the plants were capped to prevent the spread of aphids. Aphids were allowed to feed on each experimental plant for 48 hours. Plants were then sprayed with a horticultural oil solution (SAF-T-SIDE, ClawEl Specialty Products, Pleasant Plains, IL) to kill the aphids. Mock-inoculated plants received the same treatment but uninfected aphids were fed on uninfected tissue prior to being transferred to experimental plants. To test the plants for BYDV-PAV infection and to quantify relative viral titer concentration, a compound indirect double-antibody sandwich Enzyme-linked Immunosorbent Assay (ELISA; Agdia Inc., Elkhart, IN, USA) was used on aboveground tissue from experimental plants (Cronin et al. 2010).

Plants were allowed to grow for five months and then harvested. Each week the longest leaf was measured. We measured photosynthetic capacity ($A_{max}$: μmol $CO^2$ $m^{-2}$ $s^{-1}$) on the youngest, fully mature leaf of a ramet using a CIRAS-2 gas exchange analyzer fitted with a rice cuvette (PP Systems, MA, USA). At harvest, plants were separated into above- and belowground portions. Both above- and belowground biomass was placed in a drying oven. Plants were dried at 60˚C for a minimum of 72 hours to obtain dry biomass values. Soils were

frozen and stored at -20˚C until they could be processed. The belowground fraction was washed to separate roots from soil. A subset of the roots from each individual were collected before drying, stained with trypan blue following the methods outlined in (Koske and Gemma 1989) and scored for intraradical AM colonization using the magnified gridline intersect method (McGonigle et al. 1990). Using this method the percentage of root length colonized by intraradical hyphae was measured using a compound microscope (200-400x).

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