

Phenology of Arbuscular Mycorrhizal fungi and Dark Septate Endophytes across an elevation gradient

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Abstract:

Arbuscular Mycorrhizal Fungi (AMF) and Dark Septate Endophytes (DSE) participate in symbiotic relationships with host plants in the Rocky Mountains. The effects of plant-host phenology on these fungal symbionts is important in our understanding of how climate changes effects on plants will change the dynamics of this symbiotic relationship. In this observational study, I took plant-root core samples at three locations from three different plant species on three different dates throughout the growing season and recorded the AMF and DSE colonization. The data revealed that AMF colonization decreases consistently, in all elevations except the high elevation where it rapidly decreases, throughout the season, while DSE increases consistently in all sites. These results are important because they give us insight into what environmental effects dictate symbiotic fungal growth and how climate change could have implications on future seasons.

Introduction:

In the harsh conditions found on the Rocky Mountains, plants need assistance gathering valuable nutrients to survive. Many plants have overcome this challenge by entering into a symbiotic relationship with certain groups of fungi. These fungi then grow throughout the root system and exchange nutrients with the host plant. Two common fungal groups, both found in the roots of plants native to the Rocky Mountains, are the arbuscular mycorrhizae (AM) and dark septate endophytes (DSE). Although much work has been done to describe plant-fungal mutualisms, little is known about how the strength and effects of these mutualisms vary over time or across different environmental contexts.

Plants participate in a signal exchange with the fungi that allows the fungi, known as mycorrhizae, to penetrate the root cortex without triggering the plant's defenses (Helber, 2011). AM fungi are the most common and most studied fungal group that participates in symbiotic relations with plants (Bever et al. 2001). These fungi are involved with as many as 80% of vascular plant species in all major biomes (Smith, 2009). Arbuscular mycorrhizae are fungi in the phylum Glomeromycota that are obligate symbionts with plant hosts. AM fungi provide plants with phosphate and other limiting nutrients in exchange for carbon fixed into carbohydrates through photosynthesis (Parniske 2008). Plants with AM fungal symbionts are also protected from pathogens that would otherwise infect their roots (Wehner, 2010). The protection is a Mycorrhiza-Induced Resistance that is triggered by improved nutrition and a chemical signal sent out to increase a plant's metabolism (Jung, 2012). This relationship allows the plants to grow in areas that have nutrient-limited soils and pathogens that would kill the plants.

While the relationship between plants and AM fungi is reasonably well described, DSE are much less understood than AM fungi. Dark septate endophytes are a cosmopolitan ecological group of fungi from several different fungal lineages. DSE possess many characteristics in common with AM fungi, such as their ability to provide nutrients to their hosts in exchange for carbon (Jumpponen 2001), and many could be included in the same phylum as AM fungi. In addition, they increase plant biomass production, pathogen resistance, and nitrogen levels in plant tissues and have been considered by some authors as another mycorrhizal group (Jumpponen et al. 1998). Previous work has shown that DSE colonize roots in over 600 plant species, which our group hypothesizes is a great underestimate. Additionally, AM fungi and DSEs often co-occur in many plant roots.

Phenology is the study of the timing of events in organisms' life cycles throughout the seasons. The timing of phenological events in plants plays a major role in determining plant productivity and species distribution (Chuine et al. 2001). Exactly when a flower blooms affects a plant's entire reproduction cycle. If the plant blooms too early or late it may not be pollinated and produce seeds for the next year. The timing of most of these events is often triggered by the temperature. Factors such as a late snow will delay the growth of a plant until conditions are more ideal. Due to climate changes across the world, the phenology of plants has been changing (Vitasse et al., 2011). The effects of global

warming alter the snow's melting speed and causes plants to bloom at different times than before. With altered growing seasons, the implications could affect interdependent species.

In work conducted during the 2013 field season at RMBL, Jeremiah Henning found differing levels of AM fungi and DSEs across an elevational gradient. He found that AM fungi dominate the lower elevations while DSE dominate at higher elevations (J. Henning, unpublished data). The pattern he observed may either be due to differences in fungal communities at low and high elevations or differences in plant phenology when Henning did his sampling last summer. One possibility is that DSE colonization is much more prevalent earlier in the growing season, regardless of elevation, and that AM fungi begin colonizing roots a bit later in the growing season. Because Henning's sampling was conducted at the same time at all sites along the elevational gradient, he may have simply sampled the very early growing season at high elevations and mid-growing season at low elevation. If fungi are affected by the phenology of plants, the changes plants are undergoing may affect the relative abundance of different fungal species. The questions I wish to answer are: Do phenological patterns of fungal root symbionts differ by taxonomic group? Do these patterns of phenology differ along an elevation gradient?

I expected to find similar results to Henning's collected last year. I predicted the DSE fungi would dominate in the higher elevation and AM would dominate the lower. However, I expected that the difference in colonization levels would be smaller when phenology is accounted for. I accounted for this by using degree-days since onset of growing season as a factor in a repeated-measures statistical design. Because the growing season is delayed in higher elevations, the DSE may be able to use this as an advantage when competing with AM for roots. It may also be that the high elevation sites are beyond the physiological limits of AM fungi. With the change in plant phenology, the relative abundance of DSE may increase throughout the growing season at both low and high elevations.

Methods:

This study was conducted along an elevation gradient near the Rocky Mountain Biological Laboratory (Gothic, Colorado USA) during the summer of 2014 (map 1). I went to three locations over the summer to collect root samples and brought them back to Gothic to quantify their levels of fungal colonization. My lower elevation site, at 2600m, was the Almont Triangle (map 2). Seven samples each were taken of *Wyethia amplexicaulis* and *Potentilla gracilis* on May 28th, June 14th, and June 29th. These plants were chosen because *Potentilla* is found across a wide range of elevations in the region and *Wyethia* is one of the most common plants in the Almont Triangle.

The middle elevation site was south of the Elkton town site near the Pfeiler Homestead (2800m) (map 3). I collected seven samples of *Potentilla gracilis* and *Erythronium grandiflorum* on June 2nd, 11th, and 24th. The *Erythronium* was chosen because it is a common flower as well as being found in two of the three sites.

At 3400m, the higher elevation site was located at Paradise Divide near the base of Cinnamon Mountain (map 4). I collected seven samples of *Potentilla* and *Erythronium*. The dates were later for this site due to the late snow this year; the growing season did not begin until snowmelt in late June – early July.

Every plant sampled was examined for its phenological status in order to see if there is a correlation between that and the relative abundance of fungal species. To determine the phenological stage of each plant individual, I measured the plant height and counted the number of leaves and inflorescences produced. To assess mycorrhizal colonization, I collected a 2.5 x 10 cm soil-core from beneath the plant. Soil cores were transported back to RMBL where I extracted live roots from the soil core.

I cleared and stained the root samples according to the procedure outlined by Koske and Gemma (1989). I first placed samples into a 10% solution of KOH to soak in for 24 hours. Then they were rinsed off and submerged in 2% HCl for one hour. Afterwards, I soaked them in trypan blue for 12 hours. Finally, they were put in de-stain (1:1 water and glycerol solution) for two days. After the roots were mounted on slides, they were baked at 60°C for two to three days. Colonization was assessed by the grid-line intercept method (McGonigle et al. 1990) I assessed 50 locations on each slide for the presence of AM fungal hyphae and DSE hyphae as well as the presence of fungal-specific morphological structures (spores, arbuscules, and vesicles in AMF; microsclerotium in DSE).

Statistical analysis: I analyzed the fungal colonization data for each plant host species and fungal symbiont separately (six combinations total) using an ANCOVA with site as a categorical predictor variable and number of days since first collection as a continuous predictor. No transformations were necessary to meet the assumptions of the analysis. In the case of *Wyethia*, the host species was only sampled at one site, so I regressed fungal abundance on the number of days since first collection.

Results

I observed a generally high level of arbuscular mycorrhizal colonization across species and sites, and throughout time. Overall, dark septate endophytes were about 25% less abundant. When all host plant species and collection dates are combined, AMF colonization decreased with increasing elevation (Almont $.70 \pm .02$, Pfeiler $.66 \pm .02$, Cinnamon $.32 \pm .02$; mean $\pm$ standard error). In contrast, DSE colonization increased with increasing elevation across all host plants and collection dates (Almont $.35 \pm .03$, Pfeiler $.39 \pm .03$, Cinnamon $.75 \pm .01$; mean $\pm$ standard error) (Fig. 1).

The trend over time was significant in all six combinations of plant host species and fungal colonization, with AMF always decreasing throughout the season, and DSE increasing (*Erythronium* AMF $t = 4.2$ $P = .0002$, *Erythronium* DSE $t = 5.4$ $P = 5 \times 10^{-6}$, *Potentilla* AMF $t = 2.3$ $P = .03$, *Potentilla* DSE $t = 4.3$ $P = 8 \times 10^{-5}$, *Wyethia* AMF $t = 4.0$ $P = .0009$, *Wyethia* DSE $t = 5.4$ $P = 4 \times 10^{-5}$). When compared by site, there was an overall higher DSE colonization (*Erythronium* $t = 7.5$ $P = 9 \times 10^{-9}$, *Potentilla* $f = 36.6$ $P = 2 \times 10^{-10}$) at Cinnamon Mountain compared to the other sites and lower AMF (*Erythronium* $t = 7.3$ $P = 2 \times 10^{-8}$, *Potentilla* $f = 65.9$ $P < 1 \times 10^{-10}$). The site $\times$ time interaction displayed a significant difference in *Erythronium* AMF ($t = 2.2$ $P = .03$) and *Potentilla* AMF ($t = 2.2$ $P = .04$), but no such difference was

observed for DSE ($P > .05$). Cinnamon Mountain had a faster decrease in AMF over time, but the increase in DSE over time was similar for all sites (Fig. 2-4).

Discussion

I observed a strong signal of phenology in fungal root symbionts across all our study sites. At the beginning of the season, arbuscular mycorrhizal fungi dominate in all the sites, excluding Cinnamon Mountain, where DSE are generally more abundant throughout the growing season. As the season gets later, the amount of AMF goes down but the dark septate endophytes begin to take over.

My first hypothesis was that phenological patterns of fungal root symbionts differ by the taxonomic group of the host plant. The data collected showed no evidence of supporting this. The fungal colonization trends over time were similar for all host species. I also hypothesized that patterns of phenology differ along an elevation gradient, which was partially supported by the data. There was a significant difference between Cinnamon Mountain and the other two locations; in particular, the rate of change in AMF and DSE abundance was much more rapid at the highest – elevation site. . The difference may be caused by the elevation or it could be caused by the effects of late snowmelt. Cinnamon Mountain had a later snowmelt that caused the plant-host growing season to be later. This later season could have an effect on when the fungal symbionts colonize a specimen. Another explanation could be the temperature. AMF may not be able to handle the colder temperatures found in higher elevations. Further studies would need to be conducted in order to understand the exact cause of this difference.

In a previous study done by Land and Schönbeck (1991), the AM fungi followed a pattern of rapidly dominating a plant's roots at the beginning of the season but decreasing until the end of the season. Our results follow this same pattern within all of our sites. Cinnamon Mountain had a more rapid decrease in AMF but DSE followed the trend in all sites. AMF may be affected by the plant growing season in not just dominance but also in opportunity of inhabiting roots. Cinnamon Mountain may be an exception because the snow did not melt until early July and the plants were unable to grow until after that time. The season was much later than for those in lower elevations. A few other factors that could cause AMF to decrease more rapidly could be the shorter growing season, higher acidic levels in the soil, or thinner soil altogether. As I did not test for any of these factors, I have no way to positively identify the factor that dictates this change.

In conclusion, this observational study has shown that the relative dominance of AM fungi and DSE changes throughout the growing season across all host plants surveyed. This is important because it can offer insight into the effects that climate change may have on future generations of fungi and their symbiotic hosts. According to the data, arbuscular mycorrhizal fungi tend to be most common in plant roots in the earlier season and dark septate endophytes in the later. Sites in higher elevations do not follow this trend. This could be caused by the later or shorter growing season or by the colder

temperatures found at the high elevations. A global meta-analysis revealed that plant phenology is advancing a mean 2.3 days/decade among 677 species averaged over the last four decades (Walther, 2010). On top of a lengthened growing season, global circulation models for higher elevations predict an increase of 1-3°C (Maxwell 1992). If this pattern continues, plants in lower elevations will have later growing seasons and the temperature in the higher elevations will increase. This change in the plants' cycles could have large impacts on the fungal symbiotic relationships with the plants. Many of the plants cannot survive without their fungal partners. If the fungi are unable to survive in the changing climate, the plants could be affected in drastic ways.

Further studies could be conducted in order to isolate whether it was the elevation or the late season that affected the plants on Cinnamon Mountain. Perhaps an experiment on snowmelt or temperature changes in high elevation locations could give better understanding into the change of pattern.

Acknowledgements

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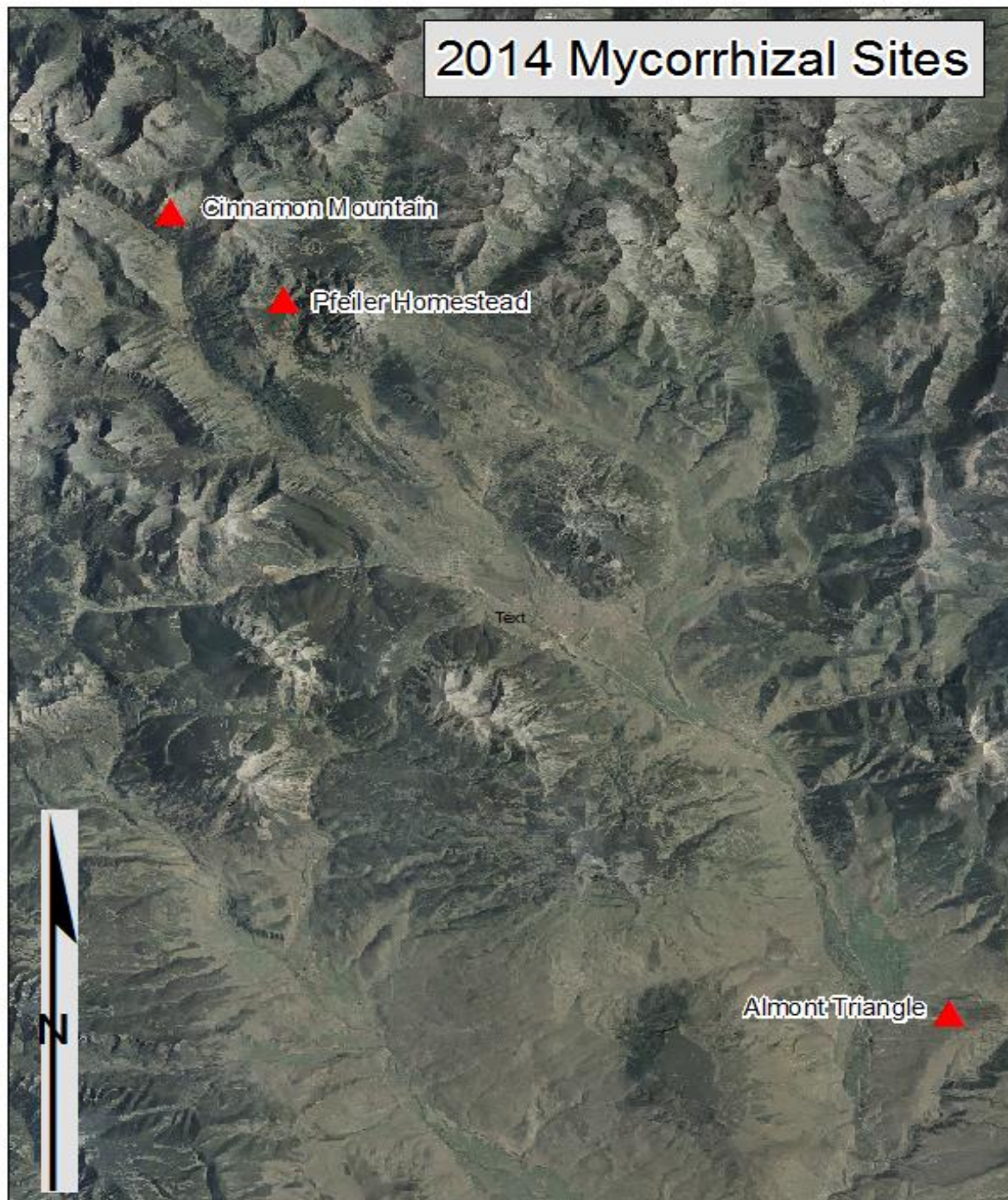
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Maps



Map 1: GIS map of all three locations used in this observational study.



Map 2: Almont Triangle with elevation and species sampled.



Map 3: Pfeiler Homestead with elevation and species sampled.



Map 4: Paradise Divide (Cinnamon Mountain) with elevation and species sampled.

Figures

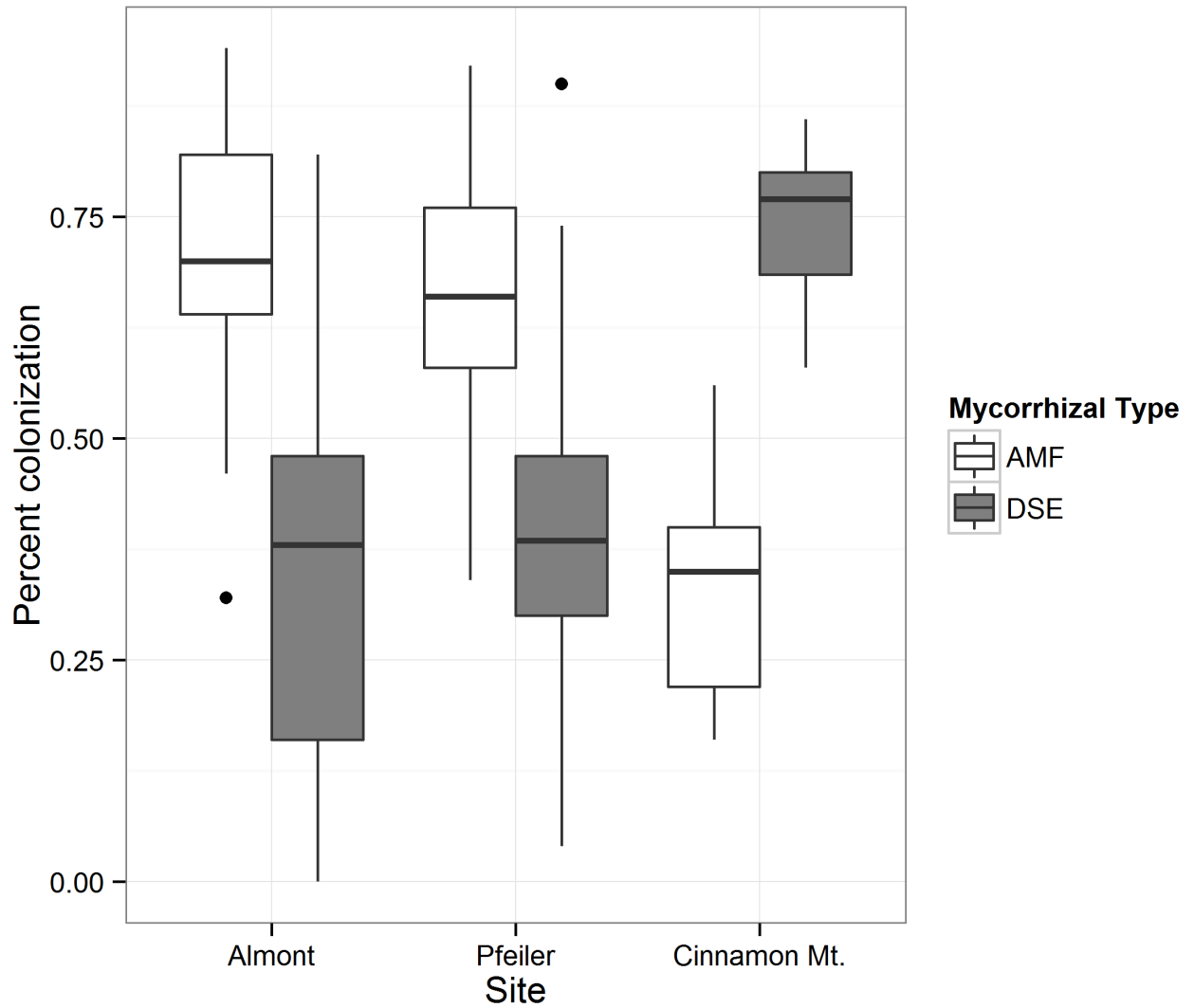


Figure 1. AMF proportional growth and DSE proportional growth compared to location, averaging all host plants and collection dates together. The central line represents the median, the boxes represent central 50% of the data points, and the whiskers represent 1.5x the interquartile range. White represents AMF and Grey represents DSE. The X axis represents the site of location and the Y axis represents percentage of fungal species growth proportionate to the number of intersects examined.

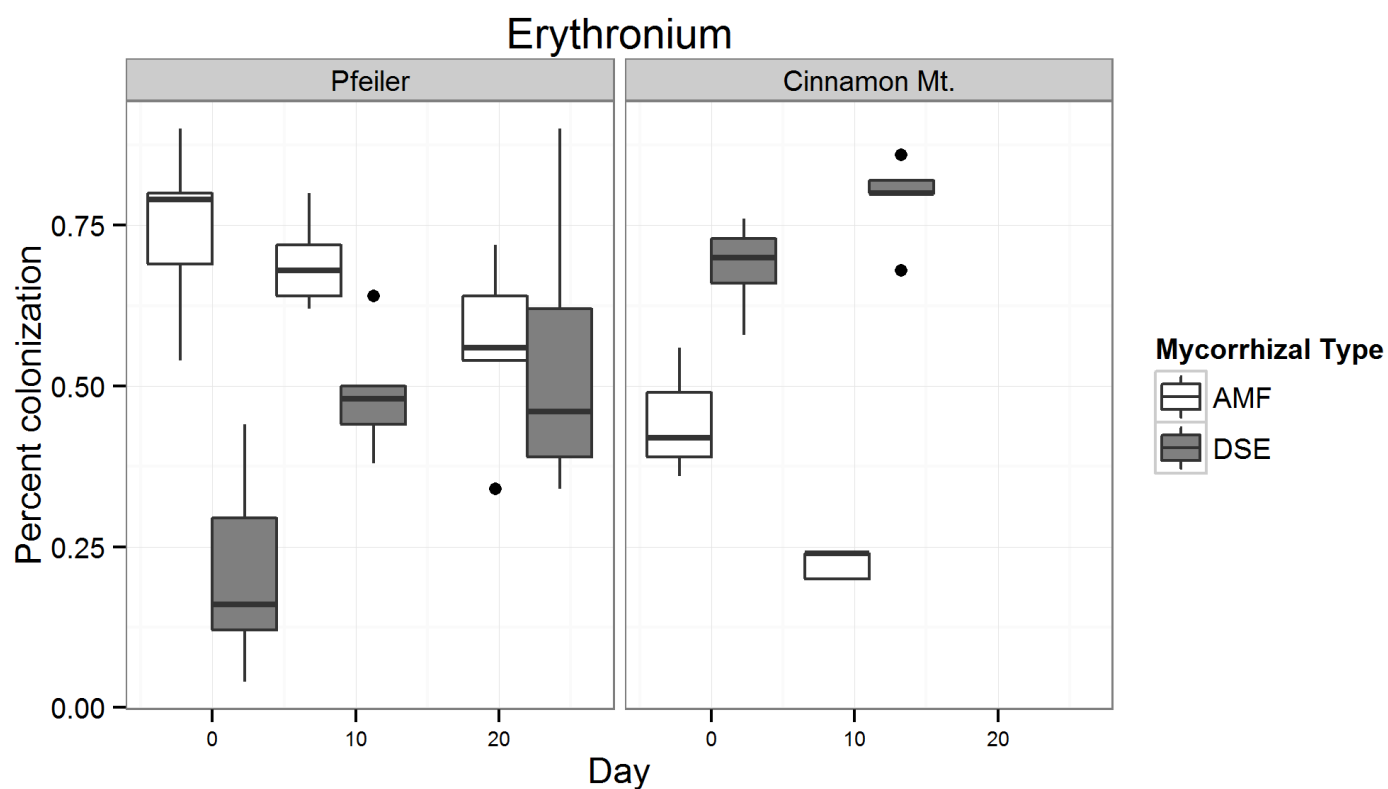


Figure 2. AMF proportional growth and DSE proportional growth in *Erythronium* compared to date and site of collection. The central line represents the median, the boxes represent central 50% of the data points, and the whiskers represent 1.5x the interquartile range. White represents AMF and Grey represents DSE. The X axis represents number of days since first collection and the Y axis represents percentage of fungal species growth proportionate to the number of intersects examined.

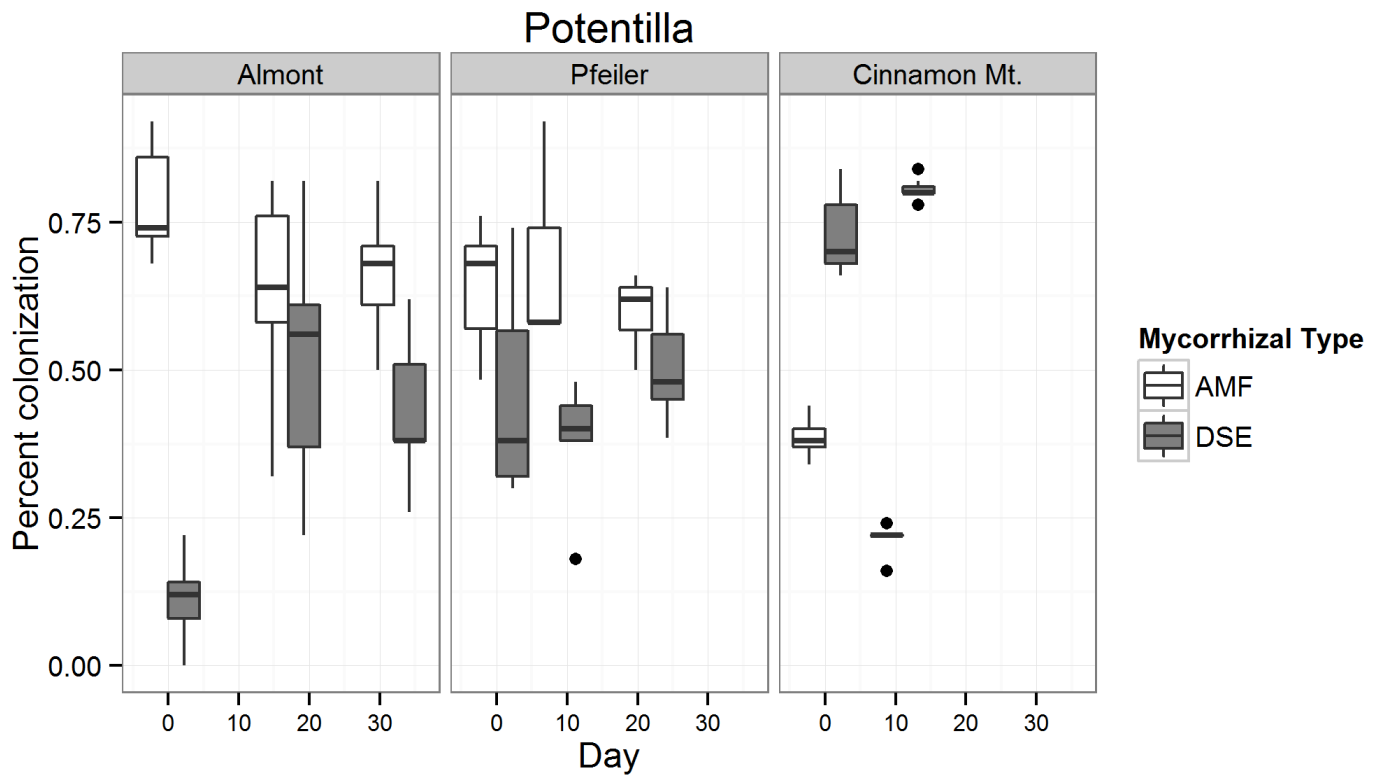


Figure 3. AMF proportional growth and DSE proportional growth in *Potentilla* compared to date and site of collection. The central line represents the median, the boxes represent central 50% of the data points, and the whiskers represent 1.5x the interquartile range. White represents AMF and Grey represents DSE. The X axis represents number of days since first collection and the Y axis represents percentage of fungal species growth proportionate to the number of intersects examined.

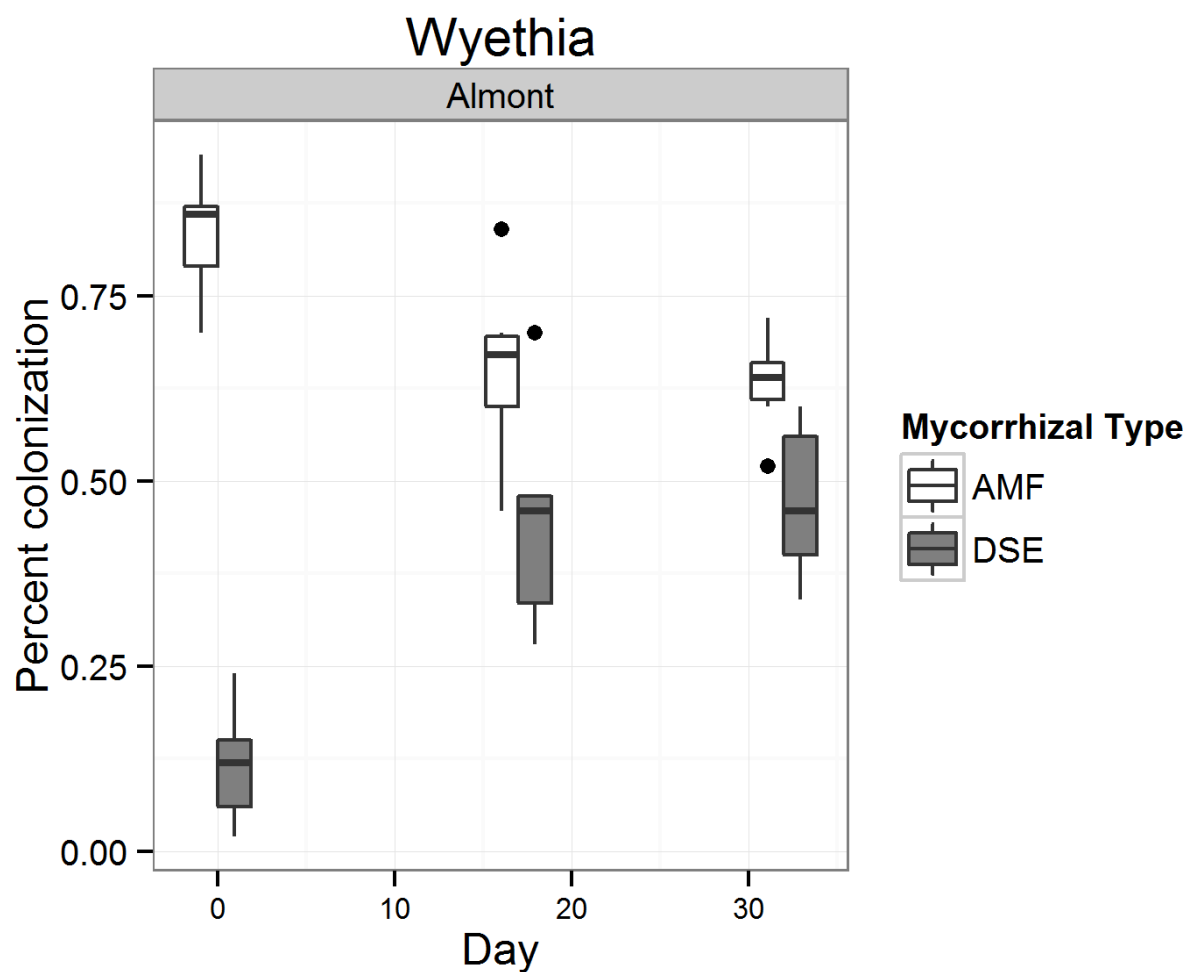


Figure 4. AMF proportional growth and DSE proportional growth in *Wyethia* compared to date of collection. The central line represents the median, the boxes represent central 50% of the data points, and the whiskers represent 1.5x the interquartile range. White represents AMF and Grey represents DSE. The X axis represents number of days since first collection and the Y axis represents percentage of fungal species growth proportionate to the number of intersects examined.

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