

The Effects of Mycorrhizal Suppression on Tallgrass Prairie Forbs

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ABSTRACT

The effects of mycorrhizal suppression by the fungicide Benomyl on the growth, demography, photosynthesis, and water relations of *Aster sericeus*, *Salvia azurea*, and *Artemisia ludoviciana* were studied at benomyl treated and control plots in watersheds 1B and 20D of the Konza Prairie Research Natural Area, Manhattan, KS from June through July of 1996. Also, end of season biomass was determined through destructive harvesting of each forb species, as well as inflorescence production and percent root colonization by mycorrhizae. Aboveground biomass was estimated non-destructively throughout the growing season using the following regression equations fit to destructive sampling on June 11 of the height, stem diameter and dry weight of 35 specimens of each species: *S. azurea* = $0.036 + 0.00069\pi(d^2/4)h$, *A. ludoviciana* = $0.037 + 0.00097\pi(d^2/4)h$, and *A. sericeus* = $0.517 + 0.78891d$, where the biomass (g), d and h (cm), and $r^2 = 0.93, 0.93, \text{ and } 0.94$, respectively. Benomyl and control samples (n=36) of each species were tagged at each site and measured on June 18, July 1 and July 18 to estimate plant biomass, and relative growth rate (RGR) over each pair of sampling dates. The plant biomasses of the control plots were significantly higher on June 18 and July 1 than the Benomyl plots. *A. sericeus* had mean plant biomasses of $0.38 \pm 0.05g$, $0.44 \pm 0.05g$, and $0.45 \pm 0.10g$ in the control plots. In the Benomyl plots the mean plant biomasses were $0.33g \pm 0.05g$, $0.42 \pm 0.04g$, and $0.45 \pm 0.04g$. *A. ludoviciana* had plant biomasses of $0.13 \pm 0.02g$, $0.12 \pm 0.03g$, and $0.16 \pm 0.03g$ for the respective times in the control plots. In the Benomyl plots the mean plant biomass for each date were $0.16 \pm 0.04g$, $0.18 \pm 0.04g$, and $0.21 \pm 0.05g$. The Benomyl treated *A. ludoviciana* had significantly higher biomass on July 1. The only significant difference in RGR between the control and Benomyl samples between June 18 and July 1 for *A. ludoviciana*. No significant differences were found between the control and Benomyl plots for photosynthesis or water potential. All three forbs were more abundant in the treated plots, significantly so in *A. ludoviciana* and *S. azurea*. Significantly more stems and stems/clone were recorded for *S. azurea* and *A. sericeus* when the mycorrhizal colonization was inhibited. This is probably due to decreased competition from dominant C_4 grasses, which are unable to thrive in the absence of mycorrhizae, allowing the forbs to become more successful. Mycorrhizal root colonization was significantly higher in the control plots for all three forbs. Inflorescence production observed in *S. azurea* and *A. sericeus* was significantly higher in the control plots on the unburned sites. The final biomass production of *S. azurea* was significantly higher in the unburned control plots and *A. Ludoviciana* was significantly higher when mycorrhizal colonization was inhibited.

INTRODUCTION

Previous studies have shown that mycorrhizal fungi enter into a mutualistic relationship with plant roots in which the fungi become integrated into the physical structures of the root (Atlas and Bartha, 1987). Mycorrhizae have been shown to perform multiple functions in plant communities. The known relationship between mycorrhizal dependency and competitive ability may influence plant growth, demography, and abundance in grassland communities (Hetrick et al., 1989). Mycorrhizae are known to exert substantial effects on plant growth and fitness, therefore their benefits should be assessed in natural conditions on a wider range of plant species. I observed the effects of mycorrhizal suppression on *Artemisia ludoviciana*, *Salvia azurea*, and *Aster sericeus*, three tallgrass prairie forbs at Konza Prairie Research Natural Area, a native tallgrass prairie.

An intimate alliance is established between plants and vesicular arbuscular mycorrhizal (VAM) fungi. In this mutualistic symbiosis the fungus is supplied with carbohydrates from the host and in return the fungi facilitate the uptake of nutrients from the soil (Hartnett et al., 1994). Mycorrhizal fungi form specialized structures, arbuscules, in plant root cells which act as the site for nutrient transfer. A network of fungal

hyphae radiate into the soil around the roots and increase the surface area, thus enhancing nutrient absorption (Newsham et al., 1995).

The widespread existence of mycorrhizal association between fungi and plant roots attests to the importance of the relationship (Atlas and Bartha, 1987). In fact, mycorrhizal fungi have been proven to be nearly ubiquitous on tallgrass prairies, such as Konza. Plants, however, may vary in their mycorrhizal dependency and responsiveness (Hetrick et al., 1994). Obligate mycotrophs, such as C_4 grasses, require the symbiosis in order to achieve maximum growth and development. On the other hand, facultative mycotrophs, such as most cool season C_3 grasses, benefit from the symbiosis but do not necessarily need it to survive. Forbs have been shown to give diverse responses, from large positive growth to negative growth (Hetrick et al., 1992). Due to these differences in plant dependency, mycorrhizae may mediate plant competition, which is an important process influencing plant growth dynamics, demography and communities in grasslands (Hetrick et al., 1994).

Although mycorrhizae are best known for their ability to improve plant-phosphorous relations, they also serve a variety of other functions. They have been shown to

improve the uptake of other nutrients such as nitrogen and copper which are important to plant survival (Newsham et al., 1995). Even when plants are capable of surviving without the mycorrhizal symbiosis, those with it require less fertilizer and withstand heavy metal and acid rain pollution better (Kendrick, 1992). Likewise, they are thought to have an increased resistance to pathogens that affect plant roots. VAM fungi have also been shown to aid plants in their defense against herbivores, possibly by altering leaf chemistry (Newsham, 1995). Plants benefiting from the mycorrhizal symbiosis are supposedly capable of tolerating high soil temperatures and salinities along with wider extremes of pH (Kendrick, 1992). The benefits of the mycorrhizal association make it possible for plants to occupy habitats they otherwise could not (Atlas and Bartha, 1987).

Previous studies have shown that mycorrhizae may powerfully impact plant competition and composition in some plant communities (Hartnett, 1994). However, few studies have focused on the influences of mycorrhizae at the plant population level. Likewise, the possible effects of mycorrhizae on demographics have not been studied extensively on co-occurring plant species in their natural ecosystem. Recently, interest in the fungus-plant relationship has dramatically increased due to potential benefits to agriculture and forestry, and to the revegetation of ecosystems damaged by human activity.

Relatively few studies have focused attention on the mycorrhizal dependency and responsiveness of forbs. Previous studies assessing forbs found coarse root systems which tend to indicate obligate mycotrophs, despite their predominantly C_3 photosynthetic pathways. However, the few studies that have been performed make generalizations about forb dependency inconclusive. Indeed, each forb observed in this study responded quite uniquely. The question as to whether mycorrhizae benefit their hosts by improving water uptake and increasing photosynthesis also remains controversial. Two studies by Lehto (1992a, 1992b) proved contradictory, one showing that plants infected with mycorrhizae take up more water and have increased photosynthesis and the other showing no differences between the mycorrhizal and non-mycorrhizal plants, although they were undoubtedly influenced by environmental conditions of nutrient deficiency and nutrient adequacy.

The objectives of this study were to observe the effects of mycorrhizal suppression on growth and demography of three tallgrass prairie forbs as well as assessing its influences on photosynthesis and leaf water potential. The study also determined the influence of mycorrhizal fungi on inflorescence production

MATERIALS AND METHODS

Two sites were selected at which to determine the effects of mycorrhizal suppression on *Artemisia*

ludoviciana, *Aster sericeus*, and *Salvia azurea*, at Konza Prairie Research Natural Area, a 3,487 hectare tallgrass prairie preserve located in the Flint Hills regions near Manhattan, Kansas. The LTER site is owned by the Nature Conservancy and managed for ecological research by Kansas State University. Vegetation is primarily native prairie grasses with a variety of other grasses and forbs. The soil type is Chase silty clay loam.

The influence of mycorrhizal suppression on growth, demography, photosynthesis, and water potential, as well as inflorescence production were examined by studying two previously established sites at Konza Prairie, each possessing 20 two by two meter plots within the natural prairie environment. The plots were randomly assigned to one of two mycorrhizal treatments. Half the plots were designated to receive a (-myc) treatment, where the mycorrhizal fungi were suppressed by the application of a fungicide, Benomyl (Benlate SODF, E.I. duPont de Nemours & Co., Wilmington, DE). Benomyl has been applied to the plots biweekly during the growing season for the previous three years. The plots each received 1.25 grams per square meter of fungicide which was added to the soil simultaneously with two gallons of water. The remaining plots, designated (+myc) represent the control plots and they received two gallons of water alone. The naturally occurring mycorrhizal activity was unaltered by this procedure.

Benomyl has been proven to successfully suppress colonization by mycorrhizal fungi and has shown no direct effects on a wide range of plants in the absence of the fungi. Also, it has been used in several previous experiments to manipulate mycorrhizal activity (Hartnett, 1993). Benomyl, however, is not specific to mycorrhizal fungi and is capable of suppressing pathogenic fungi as well. Under these circumstances, any advantage bestowed on mycorrhizal plants over fungicide treated plants may actually underestimate the benefits of the mycorrhizal association.

Initially, 35 samples of each plant species were harvested. Height and stem diameters were measured using a ruler and vernier calipers. This information allowed volume to be estimated using the standard equation for a cylinder, $\pi r^2 h$. After drying the plants in an oven for 48 hours at 60 C, the dry weights were determined and a regression line was developed. This method of measuring growth allowed biomass to be estimated non-destructively throughout the growing season. Thirty-six samples of each forb species in both the control (+myc) and Benomyl (-myc) treatment plots at each site were randomly chosen and marked. Their heights and stem diameters were measured and recorded biweekly throughout June and July.

Using estimated biomass, the relative growth rate (RGR) was calculated for each species between observation times one and two, and for the interval between times one and three, which were 14 and 28 days respectively, using the equation: $(\log \text{biomass @ Time}_2 - \log \text{biomass @ Time}_1) / (\text{Time}_2 - \text{Time}_1)$.

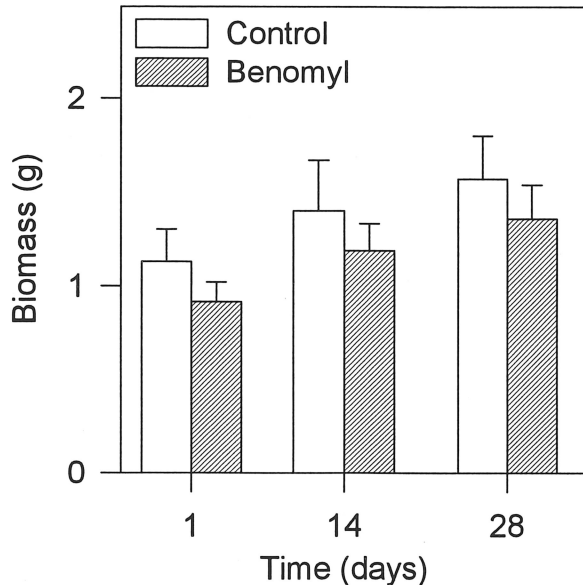


Figure 1. Mean ($\pm 1SE$) biomass of *S. azurea* in treatment and control plots at the Konza Prairie.

In another study to determine the impact of mycorrhizal fungi colonization on demography of prairie forbs, the abundances of each species were determined in each of the 20 4m² plots. The number of clones and the total stem numbers were recorded, as well as the number of stems per clone for *A. sericeus* and *S. azurea*. This allowed observations to be made on how mycorrhizal suppression influences structure and competition in a grassland community. *A. ludoviciana* clones were too difficult to distinguish accurately so only total stem number was examined.

To study the effects of mycorrhizal suppression on physiological factors, photosynthetic rate was measured with a Li-Cor (model 6200). Measurements were taken on clear days within two hours of noon. Six samples of *A. Ludoviciana* and *S. azurea* were taken from each treatment at both sites for both photosynthesis and water potential measurements. Leaves were harvested from non-tagged plants and returned to the lab where water potential was measured with a pressure bomb. *A. sericeus* was not used in the physiology study due to its small leaf size.

At the end of the growing season the final biomass production was ascertained by destructively harvesting 36 samples of each forb species from both treatments and determining their actual dry weights. Inflorescence production was also measured for forbs collected from the control and fungicide treated plots, again the sample size was thirty-six. Both flowers and buds were counted and averaged for the plots. Also, an analysis of mycorrhizal colonization of roots was performed to confirm that there was in fact a difference in treatments. Dried root samples were stained and examined microscopically to determine the percent root colonization.

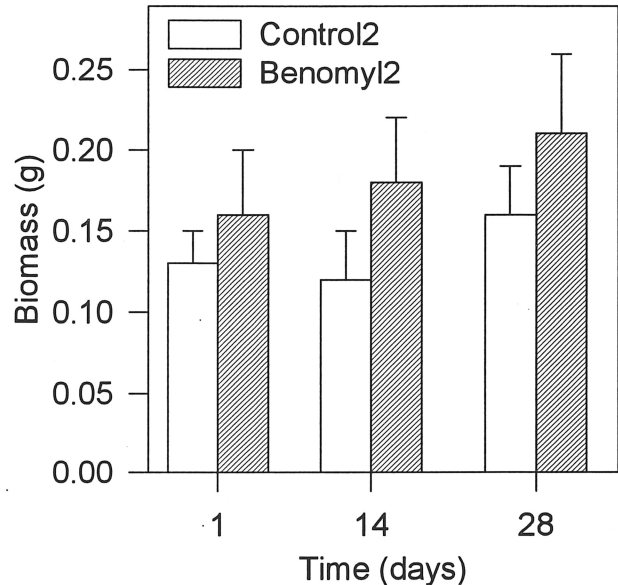


Figure 2. Mean ($\pm 1SE$) biomass of *A. ludoviciana* in treatment and control plots at the Konza Prairie.

RESULTS

The thirty-six samples of each plant species that were initially harvested were used to create a regression curve and make it possible to determine the approximate biomass of the non-destructed plants manipulated in the study. The equations used to estimate biomass were: $0.036 + (0.00069 * \text{volume})$ for *Salvia azurea*, $0.037 + (0.00097 * \text{volume})$ for *Artemisia ludoviciana*, and $0.517 + (0.78891 * \text{stem diameter})$ for *Aster sericeus*. The equations were shown to be accurate indicators of actual biomass since statistical analysis revealed r^2 values of 0.93, 0.93, and 0.94 respectively.

The effect of mycorrhizal symbiosis on patterns of growth and development varied among the three species observed. All three forbs showed unique responses to the mycorrhizal suppression by Benomyl. The mean biomass for *S. azurea* in the control (myc+) and Benomyl plots at sample times one, two and three (June 18, July 1, and July 18) are shown in Figure 1. The plants measured in the fungicide treated plots showed lower biomasses, significantly so between June 18 and July 1.

Artemisia ludoviciana showed the opposite response, and the suppression of mycorrhizal activity by benomyl increased its mean biomasses. As indicated in Figure 2 the plants in the treated plots had higher biomass at each two week interval. The only significant difference was observed on June 18, during the second measurement.

Aster sericeus was relatively unaffected by the mycorrhizal suppression as there were no significant differences in biomass at any of the measuring periods. Figure 3 shows the similarities between biomass of *A. sericeus* in the control and benomyl treatments.

The relative growth rates (RGR) that were calculated

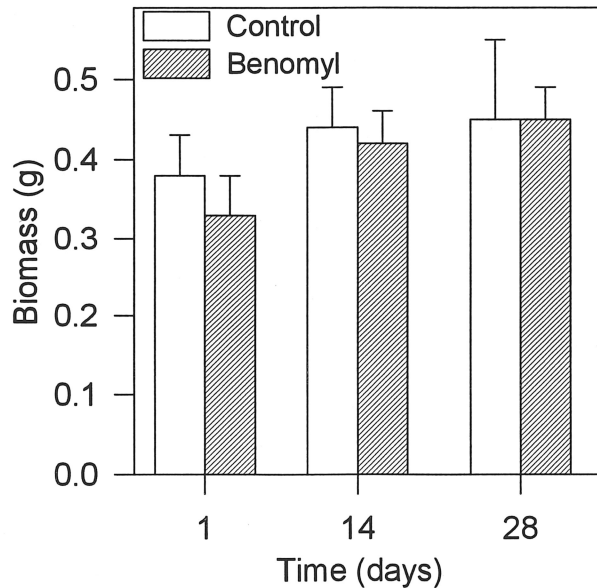


Figure 3. Mean ($\pm 1SE$) biomass of *A. sericeus* in treatment and control plots at the Konza Prairie.

in $g\ g^{-1}\ day^{-1}$ by using the estimated biomasses were determined between measurements on June 18 through July 1 and June 18 through July 18. Figure 4 presents the results obtained for all three forbs and allows comparisons in plant growth to be made between the control and fungicide treated plots. The RGR of *A. ludoviciana* was significantly higher in the benomyl treated plots between June 18 and July 1 because the plants in the control plots actually experienced a negative growth. After considering the entire 28 day period, however, there was no significant difference but the RGR of the benomyl plants remained higher. The RGR of *S. azurea* in the control plots increased slightly between June 18 and July 1 and between June 18 and July 18. The RGR of *S. azurea* in the fungicide treated plots remained constant throughout. The RGR of *A. sericeus* was similar in the control and benomyl plots between times one and two and one and three, although they both declined over the last 14 days. There were no significant differences in RGR between forbs in the control and benomyl treated plots for either *S. azurea* or *A. sericeus*.

In the demography and plant community studies significantly more *A. ludoviciana* and *S. azurea* stems were found in the treated plots, 56.5/ plot compared to 29.1 in the control plots and 65.65/ plot compared to 32.25 respectively. *A. sericeus* had 18.35 stems/ treated plot and 9.75 stems/ control plot which was not significantly different. Likewise, no significant differences were observed in the number of clones/ plot, although the fungicide treated plots were higher in both *A. sericeus* and *S. azurea*. Forb stems per clone, however, were significantly higher for both *A. sericeus* and *S. azurea* 7.3 and 13.1 in the benomyl treated plots compared to 3.9 and 8.5 in the control plots. *A. ludoviciana* clones were too difficult to distinguish

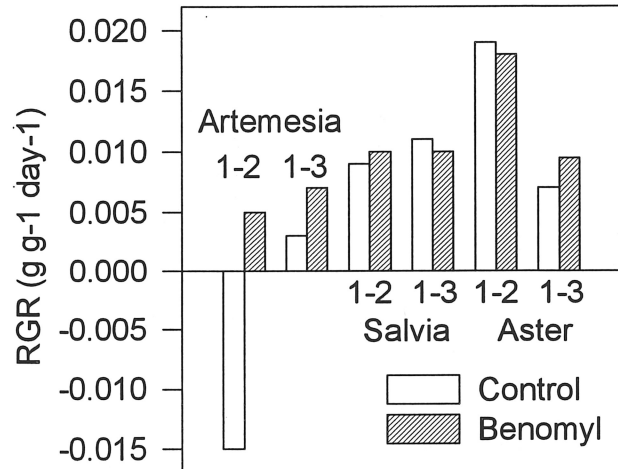


Figure 4. Relative Growth Rates of three species in treatment and control plots at the Konza Prairie

accurately so only total stem number was examined.

There was little variation in how the forbs were affected by physiological influences when comparing the control and treated plots. Both *S. azurea* and *A. ludoviciana* were observed to ascertain net photosynthetic rate and water potential. All measurements were acquired on July 3. The photosynthetic rate of *A. ludoviciana* in the treated plots was $9.55\ \mu\ mol\ CO_2\ /cm^2/sec$ and $8.37\ \mu\ mol\ CO_2\ /cm^2/sec$ in the control plots. The net photosynthetic rate of *S. azurea* in the benomyl treated plots was $15.69\ \mu\ mol\ CO_2\ /cm^2/sec$ and in the mycorrhizal plots it was $14.87\ \mu\ mol\ CO_2\ /cm^2/sec$. The mean water potential for *A. ludoviciana* receiving the benomyl treatment was 23.20 -bars while the mean of the control plants was 20.90 -bars. *S. azurea* had a mean water potential of 16.91 -bars in the benomyl treated specimens and 16.88 -bars in the plants colonized by mycorrhizae. There were no significant differences between control and fungicide treatments in net photosynthetic rate or water potential in either *A. ludoviciana* or *S. azurea*.

The examination of inflorescence production yielded differences in site and treatment since almost no flowering or budding occurred at the annually burned site. No inflorescence production was observed in *A. ludoviciana*. *A. sericeus* had an inflorescence production of .83 in the burned control plots and .52 in the fungicide treated burned plots. In the unburned plots the control plants had an average inflorescence production of 16.54 which was significantly higher than the benomyl plants that had an inflorescence production of 2.80. *S. azurea* showed the same trend. No significant differences were observed in the burned plots where the control and benomyl plants had inflorescence productions of 1.55 and 1.14

respectively. The control plots at the unburned site had and inflorescence production of 5.11 which was significantly greater than that of the fungicide treated plants which had an inflorescence production of 1.17.

The percentage of root colonization by mycorrhizae was also determined. As expected, the control plant roots were significantly more infected by mycorrhizae than the fungicide treated plant roots in all three forb species. *A. ludoviciana* was 17.6% colonized in control plots compared to 5.6% in the treated plots. Likewise, *S. azurea* and *A. sericeus* were 15.2% and 10.3% infected by mycorrhizae in the control plants and 4.8% and 2.9% respectively in the benomyl treated plants.

DISCUSSION

Certainly, the effects of mycorrhizal fungi on tallgrass prairie plant populations are important and complex. Previous studies have shown that forbs vary considerably in their growth responses to mycorrhizal symbiosis, as is further represented by the results of the present study (Hetrick, et al., 1992). *A. ludoviciana*, which demonstrated greater biomass and underwent higher relative growth rates in the Benomyl treated plots throughout the season apparently experienced a competitive release from the dominant C_4 grasses, which require the symbiosis in order to thrive. Therefore, they are less abundant in fungicide treated plots and offer less competition. This suggests a low mycorrhizal dependency since they became more successful when the competition from their highly mycorrhizal-dependent neighbors was subdued (Hartnett, et al., 1993). Thus, plant competition does in fact play an important role in grassland communities by influencing population dynamics and plant species interactions.

Salvia azurea, on the other hand, responded positively to the mycorrhizal colonization as was shown by its increased biomass in the untreated plots. There were, however, no significant differences in RGR between control and Benomyl treated subjects. The observations made on *S. azurea* showed an increased mycorrhizal responsiveness and dependency which is consistent with that of an obligate mycotroph and therefore, *S. azurea*'s growth was enhanced by its presence.

Aster sericeus, showed no preference to either the control or Benomyl treatment. This can possibly be explained by the fact that mycorrhizal infection is not always beneficial in terms of growth and nutrient uptake if the plants are already being provided with adequate nutrients (Lehto, 1992b).

All forb species had higher stem numbers, clone numbers and more stems per clone in the Benomyl treated plots. The vegetative reproduction was significantly increased in *A. sericeus* and *S. azurea* demonstrating that factors that influence growth are not necessarily the same as those that control demography. Also, plants with the mycorrhizal symbiosis may have had fewer clones and stems per

clone due to the cost of maintaining the symbiosis. If, in fact, the fungi became parasitic to the hosts and the sacrifice required to support the infection were exceeding the benefits conferred, it could have resulted in the plant having fewer resources available to devote to vegetative reproduction in the mycorrhizae plots (Lehto, 1992b). The cause, however, may also be explained by the decreased competition from the lack of dominant competitors in the Benomyl treated plots, which possibly made more resources available to the forbs growing there. This may have permitted more forbs to survive and reproduce in the absence of mycorrhizae (Hetrick et al., 1989). The clear effects of mycorrhizal suppression on forb densities indicate that mycorrhizae strongly influence population dynamics and that they can influence patterns of species coexistence, diversity, and competition (Hartnett et al. 1994).

Mycorrhizal symbiosis did not confer any advantage to the host in terms of photosynthesis and water potential. The mycorrhizal infections did not influence physiological factors in any of the forbs as had been expected with greater plant growth or biomass. This could be the result of adequate nutrient availability since Lehto (1992b) also found no differences in net photosynthetic rate when plants had access to sufficient amounts water and nutrients. Also under these conditions, Lehto (1992b) determined the water potential of mycorrhizal plants was significantly lower than those of their non-mycorrhizal counterparts. Coinciding with these results, Lehto (1992a) found that photosynthesis and water potential were higher in mycorrhizal plants than non-mycorrhizal in conditions of nutrient deficiency.

The benomyl treatment was proven to successfully suppress mycorrhizal activity in the soil as predicted in the study. Therefore, we can conclude that results were indeed influenced by an interaction between the mycorrhizae and the roots.

Inflorescence production is typically associated with changes with reproductive development (Steeves and Sussex, 1989). This indicates that *S. azurea* and *A. sericeus* in the unburned control plots had improved reproductive potential as a result of the mycorrhizal symbiosis since plants in the fungicide treated plots had poor production. *A. ludoviciana*, which demonstrated preferential growth in the treated plots, failed to flower which possibly had something to do with negative association with mycorrhizae. It is also possible for inflorescence production of reproductive parts to decrease growth below. Whether this is due to a nutritional effect or because of an inhibitor is unknown (Lydon, 1990).

Obviously, through their various roles mycorrhizae play an extremely important and influential role in shaping grassland communities. Also, there is considerable motivation to continue research in this area due to its numerous conceivable benefits to agriculture and its application to regrowth of disturbed areas, which should be valuable to humans.

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