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MYCORRHIZAL RESPONSIVENESS OF CULTIVARS AND WILD VARIETIES OF SWITCHGRASS, *PANICUM VIRGATUM* L.

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Abstract

This study focuses on switchgrass, *Panicum virgatum* L., a North American tallgrass prairie species that has been used in agriculture for only 50 years. We hypothesized that wild variants of this species are more mycorrhizal dependent than the cultivars due to selection against mycorrhizae in agricultural conditions. Mycorrhizae, naturally occurring associations between roots and symbiotic fungi, are known to benefit plants in most environments. These associations have been proposed as a means for crop improvement in agriculture. However, current agricultural practices tend to diminish the potential for crops to benefit from mycorrhizae. Two greenhouse experiments with a factorial design were performed using switchgrass inoculated or not with *Glomus intraradices* Schenck and Smith. In the first experiment, four wild varieties (PH, ONP, Ojibway and Pterophylla) and six cultivars (Forestburg, Summer, Shelter, Caddo, NU and Trailblazer) were grown for 12 weeks. From those, 3 wild types (PH, ONP and Ojibway) and 3 cultivars (Forestburg, Caddo and NU) were chosen for the second experiment. Switchgrass plants were analyzed for various physiological, root and mineral parameters in order to assess their overall mycorrhizal dependency (MD). The average mycorrhizal colonization of 37% was not different between the wild and cultivated varieties. Variety was the most significant source of variation for most of the measured parameters. It was found that each variety responded to mycorrhizal colonization by modifying different parameters. The responses of the wild group were more variable than those of the cultivated group. Forestburg, a cultivar which has not been agriculturally selected, and wild variety ONP, responded positively to mycorrhizal colonization by modifying their physiological and root parameters while mineral levels were increased in the cultivar Caddo. A cluster analysis was performed on the effect size of the mycorrhizal treatment to establish the groupings among varieties. Cluster analysis results from the second experiment

show one group with Forestburg (non-selected cultivar), ONP (wild), two varieties which improved their condition with mycorrhizal colonization, and PH (wild), which had a neutral response. The second group included NU (cultivated) and Caddo (cultivated), which had neutral responses and Ojibway (wild), which responded negatively to mycorrhizae. The MD of wild varieties in this study was lower than expected, since they showed inconsistent responses to mycorrhizae. Because of this, our hypothesis that mycorrhizal dependency of wild varieties is higher than that of cultivars cannot be strictly retained. However, the two cultivars that had undergone many rounds of agricultural selection (NU and Caddo) did not show positive growth responses to mycorrhizal colonization. This suggests that agricultural practices may have some detrimental effect on the natural ability of switchgrass to benefit from mycorrhizae. Since some switchgrass varieties clearly have positive responses to mycorrhizae, these types could be used in selective breeding programs to enhance the beneficial impact of mycorrhizae and be included in sustainable agriculture practices.

Résumé

Cette étude s'intéresse au panicum, *Panicum virgatum* L., une graminée indigène des prairies de l'Amérique du Nord qui n'est cultivée que depuis les 50 dernières années. Nous avons postulé que les variétés sauvages du panicum sont plus dépendantes des mycorhizes que les cultivars, en raison de la sélection agricole. Les mycorhizes, associations naturelles entre racines et champignons symbiotiques, sont reconnues pour bénéficier aux plantes dans la plupart des conditions environmentales. Eu égard à ces bénéfices, il a été proposé de les utiliser pour l'amélioration des plantes agricoles. Les pratiques usuelles en agriculture ne prennent pas en compte les bénéfices potentiels de la symbiose mycorhizienne, et même les contrecarrent. Deux expériences en serre ont été réalisées selon un plan factoriel avec le panicum inoculé ou non avec *Glomus intraradices* Schenck et Smith. Dans une première expérience, quatre variétés sauvages (PH, ONP, Ojibway et Pterophylla) et six sélectionnées (Forestburg, Summer, Shelter, Caddo, NU et Trailblazer) ont cru pendant douze semaines. Parmi celles-ci, 3 variétés sauvages (PH, ONP et Ojibway) et 3 sélectionnées (Forestburg, Caddo et NU) ont été choisies pour une deuxième expérience. Les plantes ont été analysées à partir de certains paramètres physiologiques, racinaires et de minéraux afin d'estimer leur dépendance mycorhizienne (DM). La colonisation mycorhizienne moyenne de 37% était semblable parmi les variétés sauvages et sélectionnées. La variété s'est révélé être le facteur le plus significatif de la réponse mycorhizienne. Il a été trouvé que chaque variété répond différemment à la mycorhization en fonction des paramètres physiologiques. Lorsque mycorhizés, Forestburg (cultivar non-sélectionné) et la variété sauvage ONP avaient des réponses physiologiques et racinaires positives, alors que Caddo (cultivar) avait une teneur accrue en minéraux. En général, les variétés sauvages avaient des réponses plus variables. Une analyse de groupement a été réalisée

sur l'ampleur de l'effet du traitement mycorhizien afin de répartir les variétés en groupes. Le premier groupe inclut Forestburg (cultivar) et ONP (sauvage) qui répondaient positivement aux mycorhizes, ensemble avec PH (sauvage) qui était plus ou moins neutre. Le deuxième groupe se compose de NU et Caddo (cultivars) dont les réponses semblaient plutôt neutres, avec Ojibway (sauvage) qui répondait de façon négative à la colonisation mycorhizienne. La dépendance mycorhizienne trouvée dans cette étude est moins élevée que celle anticipée, en raison de réponses inconstantes des variétés sauvages. L'hypothèse que les variétés sauvages ont une DM plus élevée que celle des cultivars ne peut être strictement retenue. Les deux cultivars les plus sélectionnés (NU et Caddo) se retrouvent ensemble dans le groupe qui répondait le moins positivement à la mycorhization. Ceci suggère que les pratiques agricoles ne favorisent pas et même contrecarrent la capacité du panicum à bénéficier des associations mycorhiziennes. Puisque certaines variétés de panicum ont clairement répondu de façon positive à la mycorhization, celles-ci pourraient être utilisées dans des programmes de sélection agricole pour accroître les bénéfices de la mycorhization et ainsi être incluses dans les pratiques d'agriculture durable.

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List of Abbreviations

AAC	Agriculture and Agrifood Canada, ECORC, Ottawa, ON
AM	Arbuscular Mycorrhizae (Endomycorrhizae)
ANOVA	Analysis of Variance
CDA	Canonical Discriminant Analysis
EM	Ectomycorrhizae
FM	Fresh Mass
IVDMD	*In Vitro* Dry Matter Digestibility
M+	Treatment with mycorrhizae
M-	Treatment without mycorrhizae
MD	Mycorrhizal Dependency
NU	Northern Upland
OD	Optical Density
ONP	Ontario Native Plants
PH	Prairie Habitat
PNPP	p-Nitrophenyl Phosphate
PUE	Phosphorus Use Efficiency
R/S	Root to Shoot Ratio
USDA	United States Department of Agriculture

Introduction

The agricultural sciences attempt to improve plants in order to make them more useful. Often, humans are unaware of the important processes that occur below ground and of the essential associations crop plants have with microorganisms in the natural environment. A preferable method of improving plants would be to increase plants' natural capacities to thrive in their environment, instead of altering the environment with fertilizers and tillage. Mycorrhizal associations, naturally occurring symbioses, have long been proposed as a feasible means to improve crops in agricultural systems. Unfortunately, current agricultural practices tend to discourage maximal benefits from mycorrhizal associations. It is important to assess the effect that cultivation has had on the ability of plant species to form mycorrhizal associations.

1.0 - Mycorrhizal Symbiosis

1.1– Symbiosis

Symbiosis is defined as 'the long term association of organisms of two different species'. A. De Bary, a German mycologist, was one of the first to investigate the phenomenon of symbiosis, which he defined in 1879 as 'the living together of dissimilar or differently named organisms'. These relationships span a continuum from antagonistic to mutualistic, facultative to obligate associations. Commonly, the term symbiosis is meant to imply a mutualistic relationship, but close associations can move along the symbiotic continuum depending upon the environmental conditions. Symbiotic partnerships can occur between members of all the kingdoms.

Some particularly interesting and important symbioses have occurred between members of the plant and fungal kingdoms. Because of the heterotrophic lifestyle of fungi, symbiosis as a means to acquire carbon has become a common strategy. It is estimated that a third of all fungi are involved in mutualistic symbioses (Kendrick, 1991).

1.2– Mycorrhizal Fungi

Mycorrhiza, meaning 'fungus root', is the term for associations that symbiotic fungi form with the root systems of terrestrial plants. Over 80% of plant species examined worldwide have associations with mycorrhizal fungi (Malloch et al., 1980). It has been suggested that this symbiosis has an ancient origin and that mycorrhizae have been instrumental in the colonization of terrestrial environments by plants (Pirozynski, 1980; Atsatt, 1988; Simon et al., 1993).

There exist two common types of mycorrhizae, Ectomycorrhizae and Endomycorrhizae. The ectomycorrhizae (EM) are associated with certain groups of trees and shrubs where they form a sheath around the roots. The fungal symbionts of EM, mostly basidiomycetes or ascomycetes, are very numerous, diverse and often host specific. Some EM also have the ability to live saprotrophically (Deacon, 1997), therefore are not obligate symbionts.

Endomycorrhizae are more common. They are associated with herbaceous vascular plants, some trees and non-vascular plants, the hyphae penetrate the root cortex. Some endomycorrhizal fungi are named Arbuscular Mycorrhizae (AM), because of the structures they form when in association with a host plant. Fungi of this group are

zygomycetes, order Glomales or Endogonales. There are fewer fungal species involved in endomycorrhizal associations, ~150 species are known in 7 genera, with many more hosts. The AM fungi are not host specific, although some symbiotic partnerships may be more effective than others (O'Bannon et al., 1980; Ollivier et al., 1982; Anderson et al., 1994; Graham and Abbott, 2000; Smith et al., 2000). All AM symbioses involve obligate biotrophism. Specialized types of AM, the vesicular arbuscular mycorrhizae (VAM) are restricted to the order Glomales and form distinctive vesicles inside the host root.

1.2.1– Vesicular Arbuscular Mycorrhiza Anatomy

Endomycorrhizae form structures to connect with the host plant which are primarily inside the cortex of the root. Characteristic structures of VAM are vesicles, arbuscules, large intercellular hyphae and the extraracinary hyphal network (Fig. 1.1).

The arbuscules are fungal structures that penetrate the cell wall. These finely branched structures are contained inside the cell within a periarbuscular membrane, and so never make direct contact with the plant cytoplasm (Smith and Read, 1997). The repeated branching pattern of the arbuscules is what gives them their name. They are an important site of nutrient transfer between the two partners. The large surface area created by branching makes nutrient transfer efficient. Evidence for this was presented by Rosewarne et al. (1999), who showed that in mycorrhizal plants, phosphorus transporters are preferentially located in cortical cells that contain arbuscules. Arbuscules have a short lifespan, 7-14 days. They are continually being formed in new cells and disintegrated by autolysis or digestion by host cells during the colonization (Deacon, 1997).

The other fungal structures that are found internally in the plant are the intercellular hyphae and vesicles. The more common type of AM fungi have an anatomy named 'Arum' type where the hyphae grow in the apoplastic areas. The hyphae use the space between cell walls of adjacent cortical cells to grow and expand their colonization. Some AM fungi expand by growing through the cells whey they form coils inside the cell wall; these are named 'Paris' types. The hyphae do not generally grow very far along the root and have sparse branching. The diameter of hyphae is approximately 3-4 μm (Clark and Zeto, 2000). Internal hyphae preferentially grow near the vascular bundle where phloem exudates are plentiful.

Vesicles are large ovate structures that lie intercellularly, but can equal or exceed the cell in size. These hyphal swellings are usually terminal on a hyphal branch. Vesicles are generally thought to be the energy storing structures of the fungi. They have a high lipid content, many nuclei and thick walls. It has been suggested, but not conclusively proven, that they may be able to colonize new roots (Smith and Read, 1997). A large number of vesicles would indicate a healthy plant and an efficient symbiosis.

Outside the host plant, the hyphae form a large network in the soil. This extraradical hyphal network performs various functions in the soil. The hyphae have differing diameters (2-27 μm) and wall thicknesses. They can form bridges between plants, which could be of ecological importance (Francis and Read, 1984). Hyphal networks are necessary for the survival of the mycorrhizae and are a primary source for root colonization. The small diameter of the hyphae allows access to a soil volume not available to roots alone. The hyphae can acquire minerals and water in the soil more efficiently than do roots. It has also been suggested that hyphae are able to access different sources of minerals in the soil since the biochemistry of the fungi is different from that of the plant (Tarafdar and Marschner, 1994).

Large spores are formed outside the root in the soil. Spores are lipid rich, multinucleate, and can support a germinating hypha for a week while it searches for a live root to colonize. Spores are characteristic of the species of mycorrhizal fungus. All are very large in size in comparison to hyphae or vesicles, and have thick walls, which protect against adverse conditions such as drought and cold (Smith and Read, 1997).

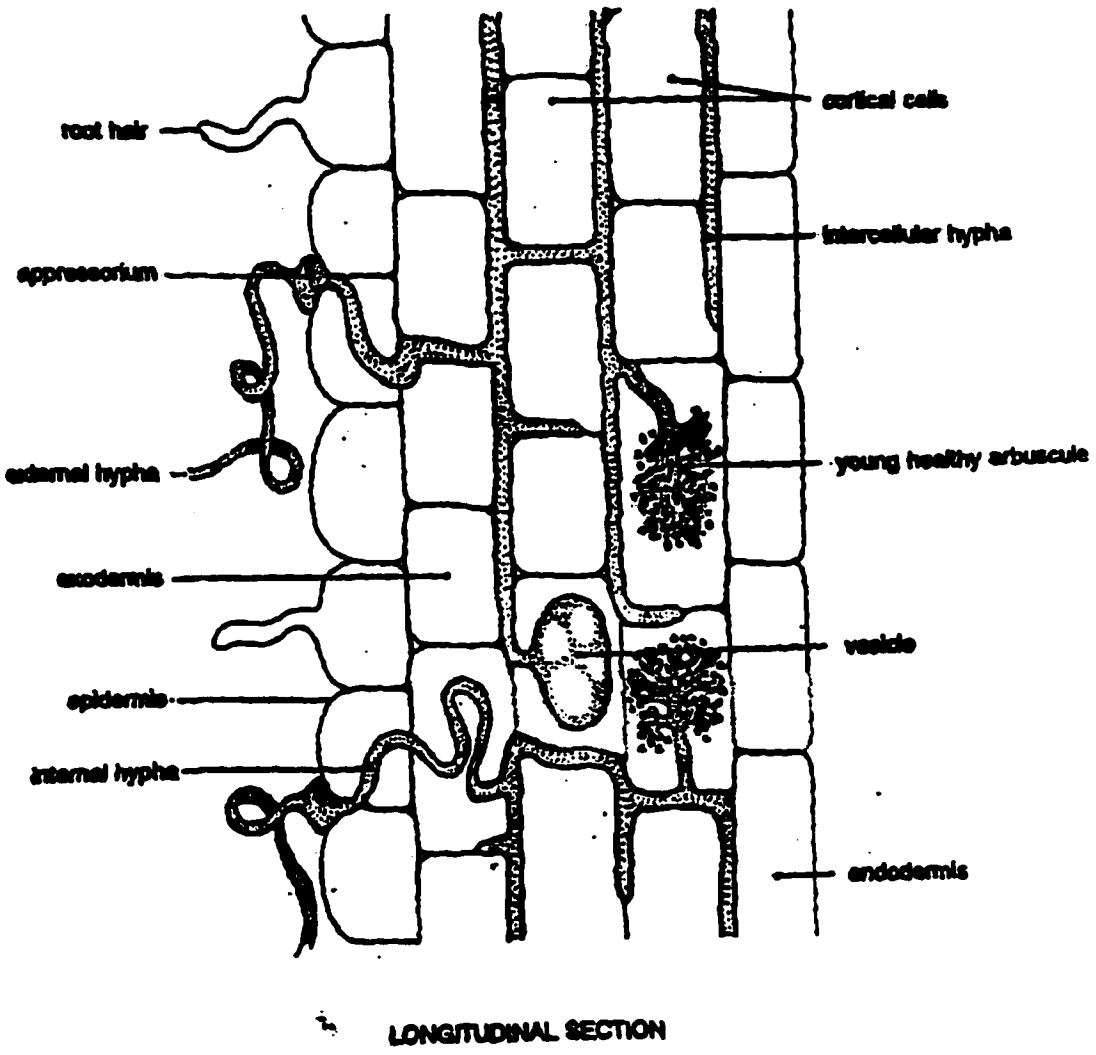


Figure 1.1. Anatomy of VAM, from Brundett et al., 1994.

1.2.2– Mode of Colonization

There are several ways for mycorrhizae to colonize roots. Spores in the soil may germinate and find their way to a live root starting a completely new colonization. Most commonly, hyphae in the existing soil network that come in contact with a root start new colonization sites. Mycorrhizal structures from previously colonized roots may colonize new roots in secondary colonization events.

It is not uncommon for a root to host several different species of mycorrhizal fungi simultaneously. Plants are able to 'control' the colonization level the roots will support and exclude new invading mycorrhizal fungi after a threshold level of colonization is reached (Vierheilig et al., 2000). Functional complementarity suggests that mycorrhizal fungal species having a structure complementary to that of the root are preferred (Koide, 2000). For example, plants with short roots prefer AM fungi which have long hyphae and can reach far into the soil while tap roots would favour finely branched fungal species.

Germinated spores or sections of the extraradical hyphal network can find their way to a root using chemical signals such as flavonoids, sugars or acids (Azcon and Ocampo, 1981; Ocampo and Azcon, 1985; Anderson, 1988; Smith and Read, 1997). Once a live hypha comes in contact with a live root, it initiates entry in the root by forming a hyphal swelling called an appressorium, which attaches to the root surface and penetrates the epidermis using hydrolytic enzymes. The fungus forms a penetration peg that forces its way into the cortex using pressure (Smith and Read, 1997).

1.2.3 –Benefits of Mycorrhizal Symbiosis to Mineral Nutrition and Stress

Growth benefits observed in mycorrhizal plants have been attributed, in a large part, to their greater mineral nutrition (Smith and Read, 1997). The most prominently researched of the elements enhanced by mycorrhizae is phosphorus (P). Many other macro and micronutrients (N, K, Ca, Mg, S, Zn, and Cu) in the soil have been shown to be absorbed at a higher rate with mycorrhizae. Mycorrhizae enhance the absorption of ions that are immobile in the soil to a greater extent than mobile ions.

A macronutrient often limiting in natural soil, phosphorus is a relatively immobile ion. Phosphates are more easily absorbed by the hyphal network, which mines the soil volume more efficiently than do roots alone because of its small diameter. Greater P content has repeatedly been found in mycorrhizal plants under both greenhouse and field conditions (Dickson et al., 1999; Schweiger and Jakobsen, 1999). Phosphates from both inorganic and organic sources were shown to be absorbed directly by hyphae in axenic cultures (Joner et al., 2000). The genes for inorganic phosphorus transport have been putatively discovered, and it seems that fungi and roots have separate P uptake transporters (Rosewarne et al., 1999).

Increased P uptake in mycorrhizal plants may be due to phosphatase enzymes. The production of acid phosphatase, which hydrolyzes the phosphate ester bond in organic sources to create more easily absorbed inorganic phosphates, may provide an additional source of P available to mycorrhizal roots (Joner et al., 2000). Roots naturally produce their own acid and alkaline phosphatases on their epidermal surface; this is supplemented

with the fungal production of phosphatases. The amount of phosphatase increases with root density and with mycorrhizae (Tarafdar and Marschner, 1994). A high proportion of the acid phosphatase produced comes from portions of the root colonized by AM fungi (Grierson and Comerford, 2000). A higher phosphatase activity has been associated with greater P efficiency in the plant (McLachlan, 1980).

Other macronutrients, (N, K, Ca and Mg) are also needed in large quantities by plants and can be limiting in the soil. Mycorrhizae have been implicated in improved absorption of N in all soil types, and of K, Mg and Ca in acid soils (Clark and Zeto, 2000). Levels of micronutrients also increase during mycorrhizal colonization. The most important uptake occurs with copper (Cu) and zinc (Zn), but iron (Fe) and sulphur (S) are also increased (Clark and Zeto, 2000). Increased absorption of Cu, Zn, Mn and Fe were shown to be dependent on soil mineral levels and P nutrition; a mycorrhizal advantage was seen at low P levels (Liu et al., 2000). Some metals, such as aluminium (Al), are taken up in lower quantities with AM, thus preventing mineral toxicity (Clark and Zeto, 2000). Heavy metals such as caesium (Cs) and strontium (Sr) can accumulate in higher concentrations in mycorrhizal plants making them useful in phytoremediation (Entry et al., 1999). Some plant/mycorrhizal fungi species are able to thrive in polluted sites; therefore mycorrhizae have been suggested as a means to aid clean up of heavy metal contamination at former mine sites (Pleger et al., 1994; Noyd et al., 1995).

Increased resistance to environmental stress factors has often been reported in mycorrhizal plants; this may contribute to observed growth or fitness gains. Resistance to

abiotic stress factors such as drought and cold, as well as biotic stress from pathogens and herbivores has been observed in mycorrhizal plants. A greater tolerance to stress has led to mycorrhizae being used in revegetation of marginal sites (Reeves et al., 1979; Noyd et al., 1995; Smith et al., 1998). Mycorrhizae can aid with water acquisition through their hyphal network. Colonized plants were able to maintain higher water potentials longer under drought conditions in greenhouse and field trials (Subramanian and Charest, 1995 & 1998; Subramanian et al., 1996; Gemma et al., 1997). Improved condition during cold acclimation was seen in mycorrhizal wheat (Paradis et al., 1995). Many studies have shown AM fungi have a deleterious effect upon pathogenic soil microorganisms (St-Arnaud et al., 1994; Filion et al., 1999; Ravnskov et al., 1999). An increase in the secondary metabolites of mycorrhizal roots has been observed (Maier et al., 1997; Peipp et al., 1997), suggesting this may be the source of biotic protection.

1.3 - Plant Response to Mycorrhizal Colonization

Environmental factors as well as genetic ones mediate efficient symbiosis, where the plant and fungus both gain the maximum increase in fitness. Soil mineral content, pH, temperature, as well as plant species, all affect the ability of mycorrhizal fungi to colonize and thrive. AM fungi are very sensitive to soil P content. In high P environments, mycorrhizae have reduced colonization rates and have even been found to be detrimental to the host plant (Manjunath and Habte, 1992; Dekkers and van der Werff, 2001).

Although AM fungi are not host specific, certain host plant- fungus combinations are functionally better than others. Symbiotic compatibility can be demonstrated by testing

many species or strains of AM fungi with one species of plant. This method has repeatedly shown that some species pairs are more advantageous than others to either the plant or the fungus (O'Bannon et al., 1980; Ollivier et al., 1982; Anderson et al., 1994; Graham and Abbott, 2000; Smith et al., 2000; Zhu et al., 2000). Some plant species are non-mycotrophic and do not allow colonization (Newman and Reddell, 1987; Tester et al., 1987). Myc- mutants, that are not able to support mycorrhizal colonization, invoke the salicylic acid defence pathway when colonized by AM fungi (Duc et al., 1989; Blilou et al., 1999).

1.3.1 – Carbon Use by Mycorrhizae

As obligate biotrophs, arbuscular mycorrhizae depend upon their host plant for all of their carbon (C) energy. It has been estimated that the fungi consume 10% of the photosynthate produced by the plant (Rygiewicz and Andersen, 1994; Tinker et al., 1994). Colonization may alter the physiology of the plant so that it diverts more of its C to the roots in order to feed the fungus, which is acting as a C sink (Rygiewicz and Andersen, 1994; Graham et al., 1997). Mycorrhizal roots receive 4-20% more photosynthate than non-mycorrhizal roots (Smith and Read, 1997). The type of carbohydrates transferred is altered so that reducing sugars such as glucose are available in the roots (Graham et al., 1997). The fungus uses glucose as its primary energy source (Solaiman and Saito, 1997), but other carbohydrates such as trehalose are also transferred to the mycelium (Bago et al., 2000). A higher rate of below ground respiration has been observed in mycorrhizal roots (Nielsen et al., 1998). However, no differences in total root C levels were noted. Carbon use efficiency has been calculated to as high as 96% in

mycorrhizal plants, due to an increase in the photosynthetic rate (Tinker et al., 1994). The amount of sugar in shoots has been found to increase in mycorrhizal maize plants (Boucher et al., 1999). The amount of C in roots is associated both with mycorrhizal dependency of the plants (Graham et al., 1997) and the level of root colonization (Schwab et al., 1991).

1.3.2 - Cost/Benefit of Mycorrhizal Symbiosis

When assessing the cost/benefit relationships of a mycorrhizal symbiosis, cost to the plant is commonly measured in carbon loss and benefit in terms of mineral acquisition. The plant also benefits by increased stress tolerance and competitive advantages in some ecosystems. These have not been included in traditional cost/benefit calculations because of the difficulty in building equations that encompass complex biotic relationships. Relative costs and benefits to the AM fungi cannot be assessed because they are obligate biotrophs and do not grow if not involved in a symbiosis.

The amount of C allocated to the fungus depends both on the host plant species or variety and the AM fungal symbiont. Whether the carbon lost represents a significant cost to the plant depends on the photosynthate available, increases in photosynthesis that may arise from increased mineral availability, and increased stress resistance conferred to the plant by the symbiosis. Monz et al. (1994) found increased atmospheric CO_2 concentration increases AM colonization in C4 grasses. This supports the hypothesis that enhanced C availability increases the amount of fungus supported by the plant. Plants are able to some extent to regulate the carbon costs that they expend on mycorrhizae (Vierheilig et

al., 2000). A model of carbon use efficiency predicts that mycorrhizal plants should have higher carbon efficiency than non-mycorrhizal plants (Tinker et al., 1994). This would suggest that the benefits of mycorrhization outweigh the costs. Douds et al. (1988) showed that there might be an optimal level of mycorrhizal colonization, since plants continue to transport photosynthate to mycorrhizal roots at a higher rate even when transport of P is not increased by the colonization. A model of cost/benefit (Fitter, 1991) proposed that the symbiosis is beneficial to plants only up to a threshold level of colonization where C costs equal P uptake by mycorrhizae. A more encompassing model of plant benefit based on mineral acquisition and % colonization was proposed by Gange (1999) (Fig 1.2). This model allows for negative responses to colonization at high soil mineral levels and predicts an optimal % colonization. This optimum depends on the environment and the genetics of the symbiotic pair. A more recent model of cost/benefit in mycorrhizae (Tuomi et al., 2001) suggests that the symbiosis is inherently inefficient in terms of C cost and is detrimental in the case of high soil mineral levels, low photosynthetic efficiency, and stress conditions.

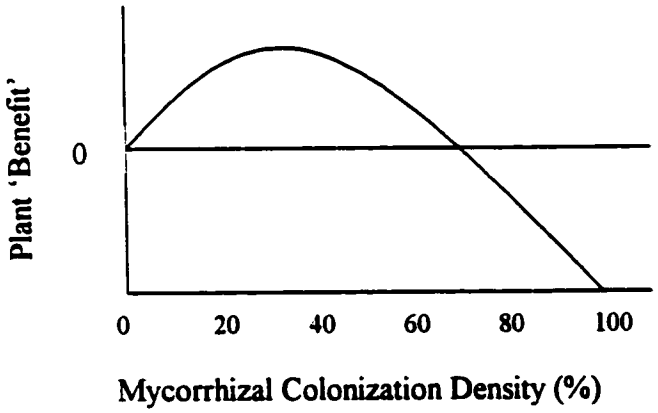


Figure 1.2. Model of plant 'benefit' and % colonization by Gange (1999).

1.3.3 – Symbiotic Continuum of Mycorrhizae

Early studies on mycorrhizae treated the symbionts exclusively as beneficial mutualists. We know that this is not always the case; host plant response to colonization can range from beneficial to detrimental. A symbiotic continuum for mycorrhizae that includes commensalism and antagonism (Fig 1.3) is included in recent studies of cost/benefit (Francis and Read, 1995; Johnson et al., 1997). These studies propose that the antagonistic condition is induced by high soil mineral abundance, carbon limitation, genotypic pairs that are not well suited, and non-native soil type.

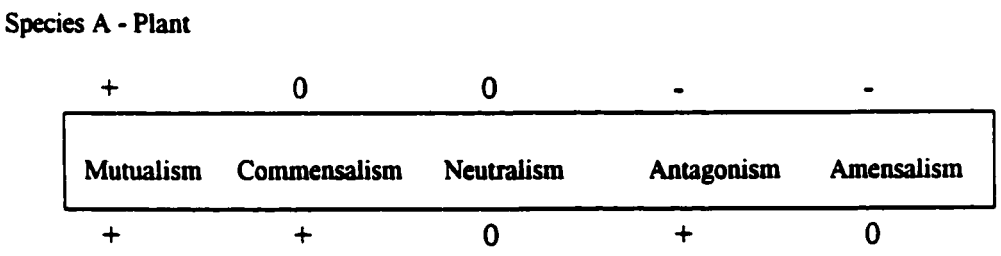


Figure 1.3. Symbiotic continuum for mycorrhizae modified from Francis and Read, (1995). +, beneficial effect; 0, no effect; - detrimental effect.

1.3.4 – Mycorrhizal Dependency

Mycorrhizal dependency (MD) refers to the amount a species or variety benefits from the symbiosis. Since 'dependency' implies a necessity and a positive response from the plant, researchers have recently proposed using the term 'mycorrhizal responsiveness' (Smith, 2000). Dependency of a species upon mycorrhizal symbiosis has traditionally been measured by the biomass increase in the host plant. The equation most often used to calculate mycorrhizal dependency is:

$$MD = [(M- NM) / M] \times 100\%$$

Where M= mycorrhizal dry mass, NM= non-mycorrhizal dry mass

Plant biomass is most commonly used to measure MD because plant mass is a good indicator of fitness, and also a parameter of much value to cultivators. It has become clear in the past three decades that plant response to mycorrhizae goes far beyond simple growth increases. Mycorrhizal dependency should take into account the directional response of the plant in factors such as root morphology, production of enzymes and hormones, increases in mineral levels and stress resistance. These factors have been shown to be influenced by mycorrhizae and could impact the plant's fitness. Interestingly, MD does not seem to be related to the % colonization of the roots (Manjunath and Habte, 1991), which was an assumption made by many researchers.

Root morphology has been used as an indicator of the MD of plants since Baylis (1970) proposed that plants with large root diameters, few root hairs and little branching would be more dependent upon mycorrhizae. If plants rely on mycorrhizae to exploit the soil area with their fine hyphae, the plant would be wasteful in producing many fine roots. Plants confined to small soil volumes, which can be adequately exploited by the root system alone, are less MD than plants in large soil volumes (Baath and Hayman, 1984). Further studies have shown that plants with large root mass, long root hairs, and small root diameters tend to be less MD (Manjunath and Habte, 1991) while increased root branching decreases MD (Hetrick et al., 1992b). Field studies of tallgrass prairie species

have shown that root architecture is a good predictor of MD, even in Gramineae which are known to have adventitious root systems (Hetrick et al., 1991; Hetrick, 1991b).

The link between root morphology and P efficiency has been found in non-mycorrhizal plants. Gahoonia et al. (1999) suggest that increased root hairs and more efficient P uptake are alternative mechanisms to dependence upon mycorrhizae. Plants that are less dependent upon mycorrhizae have higher root P absorption rates (Manjunath and Habte, 1991b & 1992; Schweiger et al., 1995). Phosphorus efficiency is not always correlated with increased growth, normally associated with MD (Smith et al., 2000). Azcon and Ocampo (1981) did not find any relationship between MD and leaf mineral content (P, N, K, Ca, Mg) as might be expected if mineral concentration and MD were directly related. Although mineral increases are seemingly a good indicator of MD, this has not yet been reliably proven. Overall, an increase in available P in the soil tends to reduce MD values (Anderson et al., 1994). Enhanced production of acid phosphatase by mycorrhizal plants may also serve as an indicator of MD (Tarafdar and Marschner, 1994).

Mycorrhizal dependency has been shown to vary between plant species and varieties (Bentivenga and Hetrick, 1992; Hetrick et al., 1992; Anderson et al., 1994). Cultivar differences have been shown in many plant species: citrus (Menge et al., 1978), wheat (Bertheau et al., 1980; Azcon and Ocampo, 1981), maize (Toth et al., 1984; Kaeppler et al., 2000), soybean (Heckman and Angle, 1987), pea (Estaun et al., 1987), cowpea (Ollivier et al., 1983), alfalfa (Lackie et al., 1988), pearl millet (Krishna et al., 1985), rye and barley (Baon et al., 1992). Many of these species have been cultivated for an

extended period of time, giving time for genetic divergence. Ecotype specificity has also been shown, MD being highest in the native soils of a particular plant (Anderson et al., 1994). Soil type specificity was shown in citrus cultivars whose MD changed depending upon the fertilization regime used (Menge et al., 1978).

Differences in mycorrhizal response among cultivars have been attributed to many factors. Mineral responsiveness has been assessed as: response to fertilizer under non-mycorrhizal conditions (Menge et al., 1978; Kaeppler et al., 2000), P uptake (Baon et al., 1992), P efficiency (Baon et al., 1993), P leaf content (Toth et al., 1984), and phosphatase synthesis (Ollivier et al., 1983). Other research examined cultivar differences in root parameters such as root length (Krishna et al., 1985), root diameter (Khalil et al., 1999), root content of reducing sugars (Ocampo and Azcon, 1985), and root starch content (Graham et al., 1997). Cultivar responsiveness may also be explained by inadvertent selection against mycorrhizae in some strains. Host plant resistance to pathogenic fungi decreased the ability of some corn or barley cultivars to be colonized by mycorrhizal fungi (Toth et al., 1990; Ruiz-Lozano et al., 1999). It is probable that all of these factors, since they are related to MD, contribute to mycorrhizal effectiveness.

The variation seen among cultivars was found to be heritable, suggesting genetic control of MD (Krishna et al., 1985; Lackie et al.,1988; Manske, 1989; Mercy et al., 1990). Recently, a quantitative trait locus that controls mycorrhizal responsiveness was found in maize (Kaeppler et al., 2000). Genetic control of MD makes it interesting from a crop breeding point of view. The concept of selection for MD has been frequently proposed

(Smith et al., 1992; Smith and Read, 1997; Manske et al., 2000). The benefit of selection for MD would be to increase plant yield and health with minimum input to the system.

2.0- Agriculture and Mycorrhizae

As the benefits of mycorrhizae became elucidated, researchers were interested in their practical applications. Use of mycorrhizae in agricultural systems was promoted as an alternative to commercial fertilization. Commercial application of AM fungi was investigated, the early focus being on finding strains of fungi that produced the greatest benefit in crop plants (Menge, 1983). Jeffries called for 'routine commercial propagative practices' in his 1987 review of mycorrhizae in agriculture. Since AM fungi are ubiquitous in soil, it was necessary to find the right type of soil in which inoculation would be useful. Tropical soils, which are often poor in mineral content, and soils damaged by mining or other contamination were cited as places where large scale inoculation might be beneficial. The new emphasis for mycorrhizae in agriculture is on selectively breeding mycorrhizal efficient cultivars and the introduction of agricultural practices, such as no-till, low-input, and crop rotation, that maintain the diversity of natural fungal populations (Hamel, 1995; Dodd, 2000).

In agricultural systems, responses to mycorrhizae have been inconsistent. Some crops show great improvement with mycorrhizae in the greenhouse (Kaeppler et al., 2000; Lambert et al., 1980) and in sterilized soil in the field (Azcon and Ocampo, 1981; Menge et al., 1978). However, in crop fields, mycorrhizal fungi can sometimes act as pathogens (Hendrix et al., 1992).

2.1- Effects of Agriculture on Mycorrhizae

Modern agricultural practices are now known to potentially have serious effects on the natural populations of AM fungi in the soil. Disruption of the extraracinary hyphal network, loss of colonization potential in fertilized soils, and host plant compatibility with fungal species all limit mycorrhizae in traditional agricultural environments. It has been shown that agricultural practices have made AM parasitic in citrus cultivation (Graham, 2000).

Tillage of the soil has been found to have negative effects on AM fungi and soil properties in general. Breaking the soil during tilling destroys the extraracinary hyphal network that is essential for colonizing new roots. Undisturbed soil maintains high mineralizeable N and acid phosphatase activity, while colonization potential, hyphal length, number of AM spores and AM species richness are reduced in soil where the hyphal network has been broken (Jasper et al., 1989; Kabir et al., 1998; Boddington and Dodd, 2000; Drijber et al., 2000). Plants in a tilled soil have been found to have lower AM colonization and lower P uptake (McGonigle and Miller, 2000; Galvez et al., 2001). No-till management has been suggested as the best method to preserve soil resources and the benefits of no-till have been linked to the presence of AM fungi (McGonigle and Miller, 2000; Mozafar et al., 2000).

Fertilized soils have less AM colonization and extraradical hyphae (Smith and Read, 1997; Liu et al., 2000). These negative effects can remain long after the applications have stopped (Dekkers and van der Werff, 2001). Nutrients supplied in organic form do not

have a negative effect on mycorrhizae and can maintain the same level of internal mineral concentration as in conventionally fertilized plants (Mader et al., 2000; Franke-Snyder et al., 2001). Organic farming, without pesticides, fungicides particularly, increases the number of AM fungi (Scott et al., 1994; M.D Smith et al., 2000). As discussed earlier, mycorrhizae can reduce fungicide and pesticide use in agriculture because of their ability to deter pathogens.

The crop species may affect the mycorrhizal populations because of host preference. AM species composition differs depending on crop species and between agricultural and natural sites (Schenck and Kinloch, 1980; Guo et al., 1993; Talukdar and Germida, 1993; Hendrix et al., 1995; Boddington and Dodd, 2000). Plant species diversity was shown to be dependent on AM fungal species diversity (van der Heijden et al., 1998a & b). Evidence suggests that fungal species diversity is also dependent on plant species despite the fact that AM fungi are considered generalists (Dhillion, 1992; Bever et al., 1996).

2.2- Cultivars and Mycorrhizae

As discussed earlier, mycorrhizal responsiveness is dependent upon genetics. In an agricultural environment, this means that some cultivars should benefit more from mycorrhizae than others. Crop breeders could benefit from the exploitation of genetic differences.

To our knowledge, no cultivar has yet been developed to maximize mycorrhizal benefit. Under intense agricultural practices, plants with high MD may have a lower yield

because the energy costs to the fungus are not balanced by increased mineral nutrition or stress tolerance. This situation would create an inadvertent selection against mycorrhizal variants. Inadvertent selection against varieties easily colonized by AM fungi has already been shown in maize by Toth et al. (1990), and this may be happening in other intensely cultivated species. It is possible that over the long term, varieties that were most responsive to mycorrhizae were discarded under high input systems and the remaining gene stock is not suitable for MD selection.

2.3- Selection in Agricultural Environments; Wild Varieties vs. Cultivars

One method of assessing the impact agriculture has had on the ability of crops to respond to mycorrhizae is to compare modern cultivars with wild varieties of the same species. Selection pressure on plants in natural environments are quite different from those imposed by human crop breeding processes, therefore wild plants should be different from their agricultural progeny. Thus far, determinations of MD in wild and cultivated variants have been done for only a few plant species (Table 1.1).

Table 1.1. Mycorrhizal dependency of wild varieties vs. cultivars for some plant species

Species	MD	References
Wheat *Triticum aestivum* L.	Wild>Cultivated	Kapulnik and Kushnir, 1991; Hetrick et al., 1992,1993
Soybean *Glycine max* L.	Wild>Cultivated	Khalil et al., 1994
Corn *Zea mays* L.	Wild>Cultivated	Khalil et al., 1994
Oats *Avena spp.*	Wild<Cultivated	Koide et al, 1988; Haynes et al., 1991
Tomato *Lycopersicon esculentum* Mill.	Wild<Cultivated	Bryla and Koide, 1990a & b

In wheat, which has been cultivated for millennia, Hetrick et al. (1992) found that wild landraces were extremely mycorrhizal dependent (55-169%) while cultivars were dependent to a lesser extent (29-100%). A genetic relationship was not found in this study as in a previous one (Kapulnik and Kushnir, 1991) where certain genomes in the ancestors of wheat were related to MD. While the first studies depended solely upon biomass to asses MD, the same cultivars were found to have different P uptake responses when mycorrhizal which were not necessarily correlated with biomass increases (Hetrick et al., 1996). So far, the evidence suggests that wheat has decreased its MD during its cultivation (Hetrick et al., 1993).

Assessments of MD, which included parameters affected by mycorrhizae such as root architecture and acid phosphatase production, were undertaken for corn and soybean (Khalil et al., 1994). This study concluded that both corn and soybean cultivars are less MD than their wild kin. Decreasing MD within an agricultural environment was not seen for all species. In oat (Koide et al., 1988; Haynes et al., 1991) and tomato (Bryla and Koide 1990a & b), cultivars were found to be more MD than the wild plants. Whether a species increases or decreases MD in an agricultural setting may depend on growth practices. Crops that are less managed, such as oats, may be at an advantage when mycorrhizal, while highly fertilized crops, such as maize, are not. According to the model of carbon efficiency of Tuomi et al. (2001), wild plants should be more MD since selective advantage disappears in commercial agriculture.

3.0- Switchgrass

Switchgrass, *Panicum virgatum* L. (Graminae; Panicoidae) (Fig. 1.4), has not been cultivated for a very long time, but its popularity is increasing, as it has many uses. Switchgrass was first used as fodder for cows and sheep and now is being developed as a potential biofuel. As a dominant grass of the native tallgrass prairie habitat, it is in demand for prairie restoration efforts. Other uses include planting for erosion control and as an ornamental plant.

Figure 1.4. Diagram of switchgrass including panicle and rhizomes.

Panicoid grasses, which are of tropical origin, use the C4 photosynthetic pathway, thus are classified as 'warm season' grasses. A native North American species, switchgrass is normally associated with the tallgrass prairie habitat and can be found across North America in a range that spans from Manitoba to Texas and from Nova Scotia to Colorado (Hitchcock, 1950). In Canada, it is principally found in Manitoba and southeastern Saskatchewan, though it can be found in patches of southern Ontario and Québec. Switchgrass usually grows to 1-2 m in height, but the native Ontarian varieties have been noted to be considerably shorter (0.5-1m) (Dore and McNeill, 1980).

Switchgrass is a perennial plant that spreads vegetatively via tillers and scaly rhizomes (a unique characteristic among panicoid grasses). Switchgrass is photoperiod sensitive and requires short days to flower. In order to produce seeds, the plants must be outcrossed, they do not self-pollinate. Switchgrass has large feathery panicles that produce small, smooth, hard seeds with poor germination rates (Looman, 1983). Seeds are often dormant and require stratification and scarification to produce good germination rates in cultivation. The shiny seeds may have had aesthetic value for the native people of North America, who reportedly used them as beads and as a component of pemmican (Dore and McNeill, 1980).

Warm season grasses have been used as summer forage because they continue to produce good fodder after cool season grasses have peaked. Switchgrass is successful in many environments and popular as an agricultural species because of its general stress

tolerance. Able to grow in acid soils, ranging from pH 4-7, switchgrass is also drought tolerant and thus has the ability to grow on marginal land (Jung et al., 1988). In Canada, cold tolerance is an issue for this grass of tropical origin. Hope and McElroy (1990) found that switchgrass originating in the Great Plains overwintered successfully due to its tough rhizomes.

One of the most desirable qualities of switchgrass to cultivators is its ability to survive in infertile soil without the need for large fertilizer inputs. Switchgrass is very P efficient; P concentrations as low as 5 mg/kg support this grass while cool-season grasses could not thrive (Moser and Vogel, 1994). In field trials, switchgrass did not increase its biomass when fertilized with P (Jung et al., 1988; Sanderson et al., 1996). Phosphorus fertilizer, if applied, is maximal at 50kg/ha (Jung et al., 1988; Staley et al., 1991; Moser and Vogel, 1994; Samson et al., 1999). Nitrogen is considered the limiting nutrient for switchgrass, and N fertilizer is often applied to switchgrass fields. Rates of N application in the field tend to be 40 kg/ha (normal), 100 kg/ha (high) and up to 180 kg/ha during experimental trials.

Recently, interest in switchgrass has focused on its possible use as a biofuel. It is proposed that fuel ethanol could be produced from cellulose derivates by enzymatic conversion to sugars and subsequent fermentation (Hopkins et al., 1995). Biofuel researchers chose switchgrass for its ability to grow in diverse environments with little input its contribution to soil conservation and revegetation of the prairies, and because of the abundance of cellulose in the stem (Sanderson et al., 1996; Madakadze et al., 1999).

A highly desirable characteristic for cultivators is the apparent tolerance of switchgrass seedlings to herbicides (Vogel, 1987), which can reduce weed competition. Resistance to toxins seems to extend to heavy metals as well. Switchgrass is able to grow normally on mine tailings that have elevated concentrations of lead and zinc without any signs of phytotoxicity (Levy et al., 1999). Some negative aspects include possible toxicity to lambs (Puoli et al., 1992), susceptibility to *Puccinia* spp and the *Panicum* mosaic virus (Moser and Vogel, 1994).

3.1- Genetics

Switchgrass has a base chromosome number of n=9. Varieties of native populations have been found that carry chromosome numbers from diploid (2n=18) to dodecaploid (2n=108) (Nielsen, 1944). In subsequent examinations of wild populations, most native accessions in the northeast prairies were found to be tetraploid (2n=36), although hexaploid (2n = 54), and octoploid (2n= 72) varieties were reported in central North America (McMillan and Weiler, 1959). Commercial cultivars are either 2n= 36, 54 or 72 (Riley and Vogel, 1982; Hultquist et al., 1996). Conflicting reports of chromosome numbers in the same variety of switchgrass (Hopkins et al., 1996) have appeared, making the issue of ploidy still relevant in this species. No morphological or physiological characteristics have been attributed solely to chromosome number.

Switchgrass has been separated into two groups based on morphological characteristics of native populations (Eberhart and Newell, 1959). The two types are now referred to as 'Upland' and 'Lowland' types and are differentiated by morphology and geography.

Upland types are found in more northern regions, tend to be fine stemmed, semi-decumbant, have a shorter growing season, and are broad based. Lowland types are southern, coarse stemmed, erect, have a longer growing season and bunched tillering. Evidence for the groups being genetically distinct as well as morphologically so, was found by polymorphisms of the chloroplast DNA (Hultquist et al., 1996).

3.2- Agricultural Selection

In order to conduct an effective selective breeding program, there must be heritable differences among populations. Variation among wild populations was established at the beginning of switchgrass cultivation (Nielsen, 1944). Estimates of the heritability of phenotypic traits were soon launched (Eberhart and Newell, 1959; McMillan and Weiler, 1959). As research continued, heritable varietal differences in switchgrass have been found for physiological parameters such as: crown node placement (Elbersen et al., 1999), leaf area index (Madakadze et al., 1998), tiller numbers and height difference (Madakadze et al., 1998b), acid soil tolerance (Hopkins and Taliaferro, 1997), response to environmental variation (Hopkins et al., 1995), % N (Talbert et al., 1983), and *in vitro* dry matter digestibility (IVDMD) (Vogel et al., 1981). Many of these traits have been selected for and incorporated into elite breeding populations. Several commercial cultivars of switchgrass have been released. Some are products of selective breeding programs (Trailblazer, Caddo, Shelter, Summer, NU) and some are native populations from which the hardiest individuals were selected (Forestburg, Cave-in-Rock).

Basis for genetic variation among cultivars being greater than variation within cultivars was recently found using molecular methods. Cultivars of switchgrass have a within population similarity of 81% while the similarity among the cultivars was 65.2% (Gunter et al., 1996). It is generally accepted among switchgrass breeders that there is high genetic variation in the species for potential crop improvements using traditional selective breeding methods (Godshalk et al., 1988).

3.3- Mycorrhizae and Switchgrass

As a C4 warm season grass, switchgrass forms mycorrhizal associations and is highly mycorrhizal dependent (Hetrick et al., 1988 and 1990). Hetrick et al. (1991) showed that C4 grasses had a much higher MD than C3 grasses in natural tallgrass prairie. Estimates based on dry biomass of wild populations from Kansas tallgrass prairie have found MD to be up to 98% (Wilson and Hartnett, 1998). Wild populations can be found with 12-50% mycorrhizal colonization (Hetrick and Bloom, 1983). Switchgrass usually shows positive growth responses to mycorrhizal inoculation, but neutral or negative responses were also found, depending on environmental conditions (Bredja et al., 1993; Entry et al., 1999).

Mineral concentrations in switchgrass were shown to increase with mycorrhizae. The minerals N and P, generally limiting in tallgrass prairie soils, showed the greatest increase in mycorrhizal switchgrass plants (Bredja et al., 1998; Johnson, 1998).

In the tallgrass prairie, mycorrhizal fungi are important determinants of the plant community composition. Competitive relationships between plants are mediated by mycorrhizal colonization. Dominant prairie grasses such as switchgrass are reduced in % cover when AM fungi are excluded (Wilson and Hartnett, 1997). Plant-fungal species preference has been noted in switchgrass. Different species of AM fungi were found to produce varying colonization levels and growth responses in switchgrass (Clark et al., 1999b). In the prairie, it was found that mycorrhizal diversity is lowest in switchgrass stands when compared with five other prairie grasses (Eom et al., 2000).

4.0- Hypothesis and Objectives

Hypothesis- Wild populations of switchgrass have a higher mycorrhizal dependency than cultivars.

This hypothesis is based on cost-benefit models and the observation that modern agricultural practices are detrimental to mycorrhizal fungi. If populations of switchgrass are in a high-input management system during selective breeding, mycorrhizae will tend towards the detrimental end of the symbiotic continuum. Therefore, plants which are less susceptible to mycorrhizal colonization will be selected and, as a result, cultivars will have a lower MD than wild plants. If conditions are more natural during the selection process, then mycorrhizae will tend towards the beneficial end of the symbiotic continuum, the largest plants will be the mycorrhizal ones, and cultivars will have a higher MD than wild plants.

The objectives of this study are:

1) To assess whether MD of wild varieties of switchgrass is different than that of cultivars.
2) To determine if there are differences in MD among switchgrass varieties.
3) To determine the impact of mycorrhizal associations on several physiological parameters in wild and cultivated varieties of switchgrass.

Materials and Methods

1.0- Switchgrass Varieties

Switchgrass was selected for this study because of its reported mycorrhizal dependency as a C4 grass species, its many agricultural uses. Because switchgrass has not been under agricultural usage for very long, cultivars may not be very genetically divergent from the wild varieties. All the varieties used in this study are of the 'upland' switchgrass type.

Genotypes of switchgrass were chosen for this experiment to represent several native populations as well as a gradient of selection pressure among the cultivars. Wild varieties were difficult to obtain since there are few harvesters of wild switchgrass seeds. It was not possible to obtain native populations from the U.S because of importation regulations. The wild varieties in this study were selected based on availability. Cultivars of switchgrass were selected based on availability from breeders, and to reflect different levels of selective breeding and ploidies.

Forestburg originates from native accessions that performed well in comparison with other switchgrass varieties. It has not undergone any selective breeding for a specific trait (Barker et al., 1988). In this way, Forestburg is similar to the wild varieties despite being a commercial cultivar. Caddo has been through selection rounds for biomass production and seed size, Shelter was selected for stalk hardiness, Summer had been selected for fungal resistance and Trailblazer had been through selection rounds for IVDMD (Alderson and Sharp, 1994). Northern Upland (NU) is comprised of a mix of many cultivars, Blackwell, Cave-in-Rock, Pathfinder and other breeder stock. Along with the

selection that was done on the cultivars that comprise NU, recurrent selection for biomass was undergone in the formation of this cultivar (C. Taliaferro, pers. comm.). A gradient of agricultural selection pressure is found in the cultivars of this experiment with Forestburg having the least and NU the most.

Of the wild varieties, Prairie Habitat (PH) is likely to be the most similar to original native populations. The seeds of this ecotype came from populations that live in the tallgrass prairie of Manitoba. This is the natural environment of switchgrass, and the population size in the prairie would be large enough to ensure good genetic diversity. Ontario populations come from very small isolated patches and they are likely to be inbred and strongly ecotypic. Ontario Native Plants (ONP) seeds come from the Toronto area, specifically High Park in the city's downtown, and the few fields still existing that contain switchgrass. Ojibway seed comes from the Ojibway Nature Centre located within the city of Windsor, Ontario. Pterophylla seeds are native to the Long Point area in Southwestern Ontario. Environmental adaptations to the particular site of these plants are likely to be emphasized in small populations such as ONP and Ojibway.

Two greenhouse experiments were performed in this study. The first experiment involved all ten varieties and had more replicates. From the results of the first experiment, six varieties were chosen (Forestburg, Caddo, NU, ONP, Ojibway and PH) for the second experiment. They were selected because they included a representative range of mycorrhizal responses, were easy to grow, represented all ploidy levels, and included

equal amount of wild and cultivated types. Changes were made to the experimental design in the second experiment, most noticeably increasing the pot size.

2.0 - Seeds

Seeds of the different cultivars used in this study were obtained from plant breeders (Table 2.1). They are either breeding stock from Universities, stock from the U.S Department of Agriculture (USDA) or from AAC. Wild seeds were obtained from native seed distributors who collect seeds from non-cultivated sources. Prairie Habitat was collected from native tall grass prairie in the Winnipeg area, Ontario Native Plants seeds were collected from the Toronto Region and Ojibway Nature Centre is a natural prairie park in the Windsor area. Pterophylla seeds are native from the Long Point region of Ontario, but the switchgrass is grown in monoculture in order to harvest the seed more efficiently.

Table 2.1. Origin of switchgrass seeds used in both greenhouse experiments.

Cultivation Level	Variety	Year of seed collection	Reported Germ %	Area of Origin	Coordinates of Origin	Distributors	Contact Person
Wild	Prairie Habitat *	1999	unknown	Winnipeg, Manitoba, Canada	49°53 N 97°09 W	Prairie Habitiats Inc., Argyle, MB	John Morgan
	ONP *	1999	unknown	Toronto , Ontario, Canada	43°39 N 79° 23 W	Ontario Native Plant Co. Downsview, ON	Charles Kinsley
	Ojibway *	1999	unknown	Windsor, Ontario, Canada	42°18 N 83°01 W	Ojibway Nature Centre Windsor, ON	Karen Cedar
	Pterophylla	1999	unknown	Long Point, Ontario, Canada	42°26 N 81°54 W	Pterophylla, Long Point, ON	Mary Gartshore
Cultivated	Shelter	1993	68%	St Marys, West Virginia, U.S	39°24 N 81°12 W	USDA New York Plant Materials Center	John Dickerson
	Caddo *	unknown	76%	Central Oklahoma, US	36° 07 N 97°03 W	Oklahoma State University	Charles Taliaferro
	Northern Upland NU 94 -1 *	1999	42%	Nebraska/ Kansas/Illinois/ Oklahoma	36°- 39° N 104° - 85°W	Oklahoma State University	Charles Taliaferro
	Forestburg *	<1998	91%	Forestburg,South Dakota, U.S	44°10 N 98°06 W	USDA North Dakota Plant Materials Center	John Dickerson
	Trailblazer	1994	67%	Nebraska and Kansas, U.S	~40° N ~ 95° W	AAC, Ottawa, ON.	Art Mc Elroy
	Summer	unknown	63%	Nebraska City, Nebraska, U.S	40°41 N 95°52 W	South Dakota State University	Arvid Boe

* - used in the second experiment

3.0- Preparation for Germination

Seeds were prepared based on the procedure detailed by Haynes et al. (1997) for germination of switchgrass. Seeds were scarified in 8 M H_2S0_4 for 5 min., rinsed with distilled water (dH_2O), surface sterilized in 5.25 % NaOCl (Clorox bleach) for 15 min. and rinsed with water. Seeds were placed one layer thick in new covered Petri dishes lined with Whatman #1 filter paper. For the first experiment plants, approximately 3 mL of 0.2% KNO_3 were added to moisten the seeds, dH_2O was used in the second experiment. Sealed Petri dishes were kept at 4 °C in the refrigerator for 14 days to stratify the seeds before removing the plates. Seeds were then transferred onto new Petri dishes with new filter papers and moistened with dH_2O. Seeds were well separated in the Petri dishes and germination was allowed to take place in the greenhouse.

Percentage germination was determined before the first experiment. Five plates of each variety received ten seeds in order to determine the % germination. After seven days, the number of seeds per plate that had emerged coleoptiles was recorded. Petri dishes were moistened daily with dH_2O. For plates that developed contamination, the germinated seeds were transferred to a clean plate. The seedlings were between 0.5 and 2 cm in height when they were planted seven days after germination began.

4.0 - Planting and Soil Preparation

First Experiment

Seedlings were planted seven days after being placed in Petri dishes for germination. The plates were randomly selected for either mycorrhizal (M+) or non-mycorrhizal (M-) treatments. If possible, plates with no contamination were used first. Each container received one germinated seed. During the first seven days of growth, if the plant died, a healthy one from the Petri plates replaced it. After seven days from the planting date, no more replacements were done and the date of plant death was recorded. Forty-nine plants per variety/treatment were planted, for a total of 998 plants.

A week after the first planting, seven more plants of each variety/treatment were planted under the same conditions as the preceding plants. These plants were kept separate and used for the root architecture analysis.

All plants were planted in SC-10 Super Cell Conetainers (Stuewe and Sons, Oregon, USA) that allowed for 164 mL of soil in 21cm depth, 3.8 cm diameter cones. Each cone was surface sterilized with Liquinox before planting. Cones were placed in RL 98 trays that allow for 14 rows of 7 cones, 98 plants in a 61cm x 30 cm tray for a total density of 528 cells/m^2 (Fig 2.1-A). Trays were rotated weekly.

Second Experiment

Seedlings were planted nine days after being placed in the Petri dishes for germination. No seedling deaths occurred. Larger pots were used in the second experiment, as the Conetainers seemed to be limiting in the first experiment. The 500 mL pots were surface sterilized with Liquinox prior to planting. Ten pots of the same variety/treatment were contained in each tray. Trays were rotated as a unit in the greenhouse once a week. Twenty plants per variety/treatment were planted (with the exception of 10 for Ojibway) for a total of 220 plants (Fig 2.1-B,C,D).

5.0 - Soil Medium

Medium consisted of a 1:1:1 ratio of potting soil: vermiculite: moistened sand (Ritchie's Feed and Seed, Ottawa, ON). This mixture was found to have a pH of ~ 4.5 before planting. All substrates were autoclaved separately for 45 min. at 120 °C and allowed to cool for 2 weeks before planting.

In the first experiment, cones were filled with soil mixture to 2 cm from the top, then approximately 0.5 cm (5-10 mL) of mycorrhizal inoculum or non-mycorrhizal control substrate was added to each cone and 0.5-1cm of soil mixture was added to top up the cone. Non-mycorrhizal treatments were prepared first in order to prevent contamination.

In the second experiment, pots were filled 1/3 with soil mixture, then 200 mL of inoculum or non-mycorrhizal control substrate was added and an additional 1 cm of soil topped the pot. Non-mycorrhizal treatments were again prepared first in order to prevent contamination.

The mycorrhizal inoculum consisted of a commercial strain of *Glomus intraradices* Schenck & Smith. (DAOM 181602) (Mycorhize Pro, Premier Tech, Rivière-du-Loup, QC). Premier Tech also supplied the non-mycorrhizal control substrate that contained filtrate of the rhizosphere microflora while excluding fungal spores. This was added to the non-mycorrhizal cones in the same manner as the mycorrhizal inoculum. Mycorhize Pro was obtained 2 weeks before planting and stored at 4 °C.

The fertilizer consisted of a low phosphorus, modified ¼ Hoagland solution adjusted to pH 6 ($NaH_2PO_4 \cdot 2H_2O$, 0.25 mM; KNO_3, 1.5mM; $Ca(NO_3) \cdot 4H_20$, 1.0 mM; $MgSO_4 \cdot 7H_2O$, 0.5mM; FeEDTA, 62.5 µM; $MnSO_4$, 2.29 µM; H_3BO_3, 11.56 µM; $ZnSO_4 \cdot 7H_2O$, 0.19µM; $CuSO_4 \cdot 5H_2O$, 0.08 µM; $H_2MoO_3 \cdot H_2O$, 0.028 µM).

6.0 - Growth Conditions

First Experiment

Seedlings were planted on April 1st (M-) or 2nd (M+) 2000 and harvested June 26 and 27, respectively, after 12 weeks of growth. All plants were grown in the greenhouse under natural light conditions. Light measurements were taken with a LiCor L.I. 185B light

meter unit on May 24 at 1 PM. Measurements were on average 1750 micromoles $M^{-2}sec^{-1}$ in the light and 600 micromoles $M^{-2}sec^{-1}$ in the shade. Plants were rotated clockwise in the greenhouse every week. Each cone was watered daily with distilled water. The ¼ Hoagland solution (20 mL/ plant) was added on April 5, May 3, 17, 30, and June 10.

Second Experiment

Seedlings were planted on August 25, 2000 and harvested on October 24 (M-) and October 25 (M+) after 10 weeks of growth. Plants were harvested early to prevent them from entering the flowering stage. Plants received natural light and 12h of light from sodium lamps. Light measurements were taken with a LiCor L.I. 185B light meter unit September 6th at noon. Average measurements were 1330 micromoles $M^{-2}sec^{-1}$ in the light and 400 micromoles $M^{-2}sec^{-1}$ in the shade. Each pot was watered daily with dH_2O. Trays were rotated clockwise in the greenhouse weekly. Pots were fertilized on August 30, September 13, 27, and October 11, with 20 mL of the ¼ Hoagland solution.

6.1- Growth Measurements

Plant height was measured weekly during the first experiment. The same 14 plants of each variety/treatment were measured. Height from the basal knot to the tip of the tallest leaf was recorded, including only live tissue. Height was measured only at harvest in the second experiment.

6.2- Chlorophyll Determination

Chlorophyll content was determined for the first experiment plants only, as chlorophyll differences were deemed not to be important. Greenness numbers were taken on June 14th2000, using a Minolta SPAD 502 handheld chlorophyll meter (provided by Dr. Malcolm Morrison, AAC, Ottawa, ON). Seven plants were chosen from each variety/treatment for chlorophyll determination. The top three leaves that were completely unfurled were used for chlorophyll determination. A minimum of five points were taken on each leaf, and these were averaged to give a leaf greenness number. Because traditional chlorophyll determination is destructive, samples were taken from Cave-in-Rock switchgrass not used in the experiment in order to create a standard curve for chlorophyll/greenness number. Samples of leaves representing various greenness number readings were assayed for chlorophyll content. Weighed leaf pieces were submerged in 1 mL of 95% ethanol and refrigerated for 14 hrs before the absorbancies at 663 and 645 nm were read on a NovaSpec 2 spectrophotometer. Chlorophyll content was determined using the following equations (Bruinsma, 1963):

$$\text{Chl a} = (\text{OD}_{663} \times 12.7) - (\text{OD}_{645} \times 2.7)$$

$$\text{Chl b} = (\text{OD}_{645} \times 22.9) - (\text{OD}_{663} \times 4.7)$$

$$\text{Total Chl (a+b)} = \text{Chl a} + \text{Chl b}$$

The graph of Chl a+b/ mg tissue vs Greenness number was used to determine the chlorophyll content associated with the chlorophyll meter readings. (See Appendix).

7.0 - Harvest

Plants were harvested over 3 days June 19 to 21, 2000 for the first experiment. For the second experiment plants were harvested on Oct 24 (M-) and Oct 25 (M+) 2000. Plants were harvested during the vegetative stage before entering reproductive stage as described by Moore et al. (1991). Roots of the plants were shaken free of soil substrate and rinsed with water in order to remove any remaining substrate. Height was measured from the basal knot to the tip of the tallest leaf. Tiller numbers were recorded. A tiller was considered to be a photosynthetic vegetative shoot arising from either an underground rhizome or a distinct branching of the stalk at the base. Fresh weights of the plants were taken first as a whole, then roots and shoots were weighed separately. The root sections of the first 10 plants of each variety/treatment were used for determination of acid phosphatase level and mycorrhizal colonization %.

Fresh plants were put in a –80 °C freezer within 2 hours of harvest. Frozen material was lyophilized for 24 hrs (Virtis UNI-TRAP freeze dryer model 10-100, Virtis Co, Gardiner N.Y, US). Dry weight was subsequently measured for root and shoot sections. Dry material was ground to powder in a modified coffee bean grinder (CBM100, Black and Decker Canada Inc, Brockville, ON) with the root and shoot sections remaining separate.

8.0 - Acid Phosphatase Determination

The method used for acid phosphatase determination was derived from McLaughlan (1980). Immediately after being removed from the soil and washed, 0.5g of fresh roots were immersed in 25 mL of phosphate solution [acetic acid buffer (0.2 M sodium acetate-acetic acid made to pH 5) containing 12.5 mg p-nitrophenyl phosphate (PNPP)]. The bottles were kept in the dark for 60 min. at 20 °C. After incubation, 5 mL from the buffered PNPP solution was removed, then titrated to pH 11 with 2N NaOH and brought to a 50 mL volume. A yellow colour appears when the solution becomes basic and the optical density of the solution was read at 400 nm. The colour is stable for 24 hrs. The blank consisted of acetic acid buffer and PNPP without root material. Results are reported in OD 400 nm/g fresh root weight/hr. Ten root samples per variety/ treatment were assayed for acid phosphatase.

9.0 - Root Staining and Percent Colonization Determination

Fresh roots were washed and patted dry. Approximately 0.5g of root material were covered with 2.5% KOH and heated in a hot water bath at 90°C for ~ 10 min. until bleached. Roots were drained, washed with dH_2O and allowed to soak in 1% HCl overnight. The acid was drained and replaced by Aniline Blue staining solution (50 mL 1% HCl, 450 mL dH_2O, 500 mL glycerol, 0.5g aniline blue). Roots were stained by heating in a hot water bath for 3-4 min. and then placed in destaining solution (50 mL 1%HCl, 450 mL dH_2O, 500 mL glycerol). Ten 1 cm root segments/plant were lined up per slide. A polyvinyl alcohol-lactic acid-glycerol medium, (PVLG- Polyvinyl alcohol

8.33g, dH_2O 50 mL, Lactic acid 50mL, Glycerol 50 mL) was used as a preservative and the slide was sealed with clear nail polish.

Percentage colonization was determined by counting mycorrhizal structures on the root segments with a light microscope at 100X and 400X magnification. A root section was considered mycorrhizal if it contained any of the structures associated with *Glomus intraradices*, intercellular hyphae, vesicles or arbuscules (Fig 2.2). Ten plants per variety/ treatment were used to determine colonization. Twenty root sections were observed for each plant (2 slides). A representative number of non-mycorrhizal plants from each of the treatments were observed to verify that no contamination occurred.

10.0 - Root Architecture Determination

The Winrhizo™ Image Analysis Program (Régent Instruments, Québec, QC) was used to determine root architecture. This method has been assessed as reliable to 200 cm root length (Bauhus and Messier, 1999). Fresh roots were scanned (HP DeskScanII, maximum dpi 2400) and then the image analyzed by the Winrhizo™ program. If the root mass was very large, it was broken into 2 parts from the tip, each were scanned separately and the results combined on a spreadsheet. The program was provided for use in the lab of Dr. Yolande Dalpé, AAC, Ottawa, ON.

11.0 – Analysis of Carbon and Nitrogen

Combustion and gas chromatography were used to determine the % of carbon and nitrogen in the dried plant samples. This was done with an Elemental Analyser (Perkin Elmer Series II 2400, Foster City, CA, US) at the JJ Hatch Isotope Laboratory, University of Ottawa. Approximately 4 mg of dried tissue was used for each sample. Three samples of root and shoot portions were measured per variety/treatment. Samples each contained the combined dried ground material of 3 plants.

12.0 - Analysis of Other Macronutrients

Macronutrient analysis was done by ICP-AES (Spectroflame, Analytical Instruments, 1994) at the laboratory of Dr. Henri Dinel, AAC Ottawa, ON. Approximately 500 mg of dried sample were digested in 20 mL of HNO_3 and 8 mL of $HClO_4$ overnight, then placed on a 90 °C hot plate. This solution was allowed to simmer overnight, then 10 mL of HF was added and the solution transferred to a 200 °C hot plate until it turned clear. If there was no residue, the liquid was boiled down to ~ 2 mL and made up to 25 mL with deionized water. The solution was then analyzed by the ICP-AES for K, P, Ca, Mg. Samples consisted of the dried powder of 3 plants combined. Three samples were analyzed per variety/treatment for both root and shoot portions.

13.0 - Chromosome Count

The number of chromosomes for each variety was determined microscopically on dividing root tips by Ms. Svetlana Kritenko in the lab of Dr. George Fedak at AAC, Ottawa, ON. Roots were placed in ice water to arrest division, then fixed in a 3:1 alcohol acetic acid mixture and hydrolyzed in 1N HCl at 60° C for 10 min. Roots were stained with Felgin stain and observed under a light microscope.

14.0 - Statistical Analyses

Statistics were done using the SYSTAT 9 program (SPSS, 1998). Two-way ANOVAs were done to compare the treatment effects with variety and cultivation level for each measured variable. When necessary, the non-parametric Kruskall-Wallis test was done instead. If an interaction was found between variety and treatment, a Tukey's test was performed to determine varietal differences. If treatment was significant with an interaction, t-tests were performed on each variety to assess mycorrhizal treatment effect.

A cluster analysis was performed on the varieties to determine their relationship to each other with regards to mycorrhizal effect. The effect size of the mycorrhizal treatment was determined by finding the difference between the mycorrhizal and non-mycorrhizal averages and dividing by the total standard error, which includes both mycorrhizal and non-mycorrhizal samples.

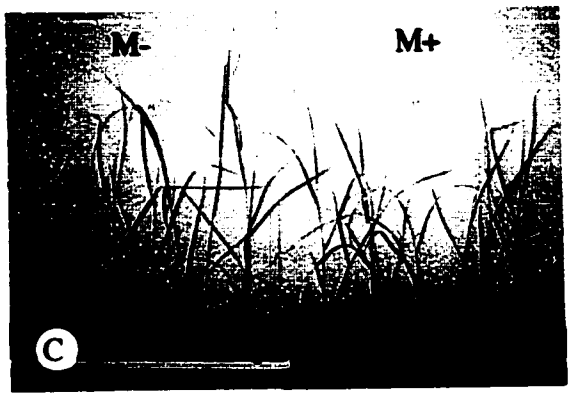
$$\text{Effect Size} = \frac{\text{Mycorrhizal Average} - \text{Non-Mycorrhizal Average}}{\text{Total Standard Error}}$$

Effect size was calculated and included in the cluster analysis for: height, tiller numbers, total fresh weight, total dry weight, dry root weight, dry shoot weight, R/S, acid phosphatase level, total root length, average root diameter, number of root tips, % small roots, % large roots, % N shoot, % N root, % C shoot and % C root. For the second experiment, macronutrient concentrations of P, K, Ca, and Mg for both root and shoot sections were included in the analysis. All six types of hierarchical cluster joining (Single, Complete, Average, Median, Centroid and Ward) were performed. The most representative and statistically valid test, Ward joining was selected for both first and the second experiments. Stepwise Canonical Discriminant Analysis (CDA) was performed to determine cluster significance.

Figure 2.1 (A-D). Switchgrass plants. A) Experiment 1 growth conditions. B) Experiment 2 growth conditions. C) The wild variety Ojibway M- and M+ plants. D) The cultivar Forestburg M- and M+ plants.


A


B

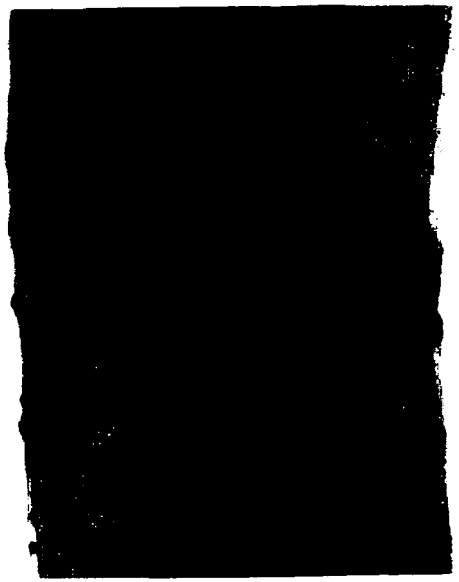

M-
M+
C


M-
M+
D

Figure 2.2 (A-D). *Glomus intraradices* colonization. A) A heavily colonized root portion seen at 100× magnification. B) Colonized root showing intercellular hyphae and vesicles seen at 100× magnification. C) An arbuscule shown at 400× magnification. D) A vesicle seen at 400× magnification.

A

B



Results

1.0 - Germination

The cultivars of switchgrass had a significantly higher germination percentage than the wild varieties (t-test, p= 0.000) (Table 3.1). There was a significant effect of variety on germination % (one-way ANOVA, p=0.000). Forestburg and Caddo had very high germination rates while Pterophylla and ONP were poor germinators. The wild varieties, except Pterophylla, had husks attached that may hinder seed germination while the cultivars were bare.

2.0 - Chromosome Numbers

Switchgrass has a base number of 9 chromosomes and is commonly found in the tetraploid (2n=36), hexaploid (2n=54) and octoploid (2n=72) forms. The most recently published chromosome counts, and the chromosome numbers we observed are reported in Table 3.2. Wild varieties in this study were all found to be hexaploid. Caddo and Northern Upland were reported to be octoploid, but only 54 chromosomes were counted in these cultivars. Forestburg and Summer had 36 chromosomes as expected. Shelter was correctly reported to be octoploid.

Table 3.1. Germination percentage ± standard error of switchgrass seeds.

Cultivation Level	Variety	% Germination	
Wild	Prairie Habitat	54 % ± 1.7	abcd
	ONP	18 % ± 1.0	cd
	Ojibway	26% ± 2.2	bc
	Pterophylla	16% ± 1.4	d
Cultivated	Shelter	62% ± 1.5	ab
	Forestburg	80% ± 1.1	a
	Summer	44% ± 2.2	abcd
	Caddo	74% ± 1.2	a
	Trailblazer	60% ± 1.7	ab
	Northern Upland	58% ± 2.0	abc
Wild		29%	β
Cultivated		63%	α

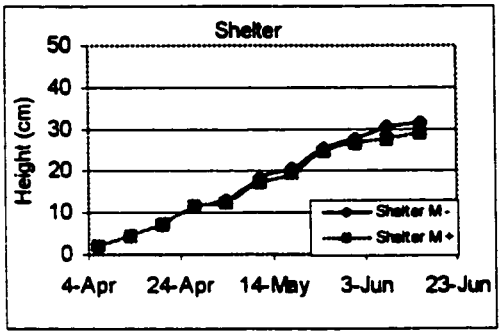
Significant differences between varieties as determined by a Tukey's test at $\alpha = 0.05$ following a one-way ANOVA at $\alpha = 0.05$ indicated by different letters.
Significant differences between cultivation level as determined by a t-test at $\alpha = 0.05$ indicated by α, β.

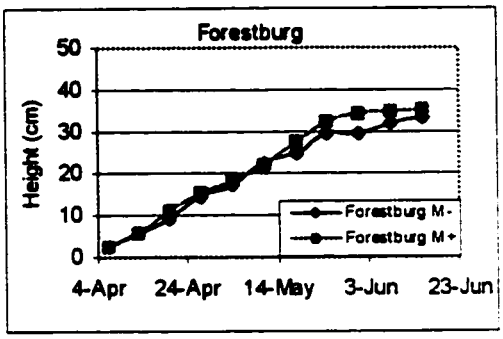
Table 3.2. Chromosome numbers in switchgrass varieties.

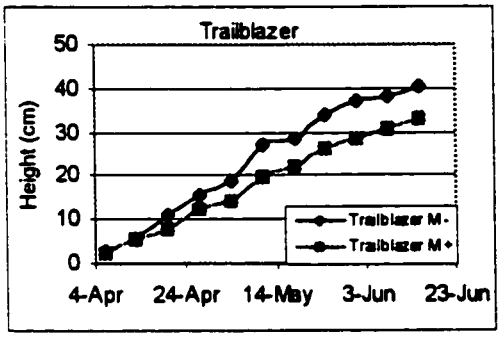
Cultivation Level	Variety	Ploidy	Previously Reported Ploidy
Wild	Prairie Habitat	54	-
	ONP	54	-
	Ojibway	54	-
	Pterophylla	54	-
Cultivated	Shelter	72	72ϕ
	Forestburg	36	36π
	Summer	36	36$\phi\gamma$
	Caddo	54	72ϕ
	Trailblazer	54	54γ
	Northern Upland	54	72λ

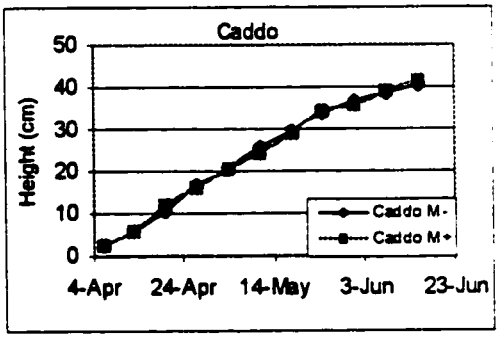
ϕ - Hopkins et al. (1996)
γ - Riley and Vogel (1982)
λ - C. Taliaferro (personal communication)
π - Elbersen et al. (1999)

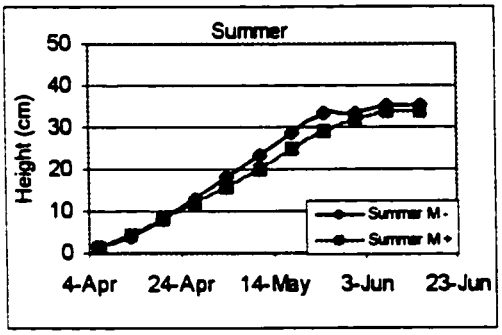
Figures 3.1-3.10. Growth rate as measured by plant height. Graphs show weekly height from basal knot to tallest leaf tip for 14 plants from each variety/ treatment. M+ plants are indicated by ■ and M- plants with ♦. Cultivars are Shelter, Forestburg, Trailblazer, Caddo, Summer and NU. Wild varieties are ONP, Pterophylla, PH, and Ojibway.

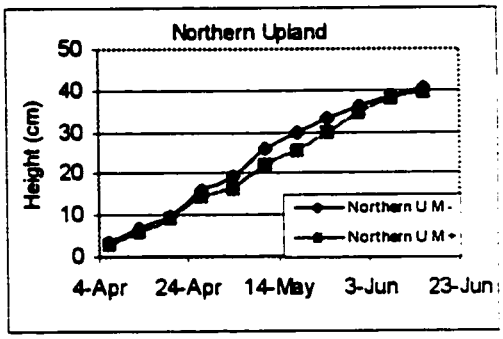

Shelter
Height (cm)
50
40
30
20
10
0
4-Apr
24-Apr
14-May
3-Jun
23-Jun
Shelter M-
Shelter M+

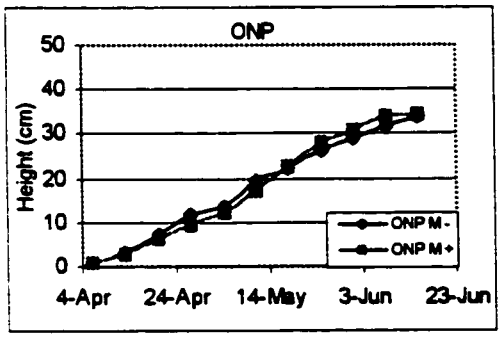

Forestburg
Height (cm)
50
40
30
20
10
0
4-Apr
24-Apr
14-May
3-Jun
23-Jun
Forestburg M-
Forestburg M+

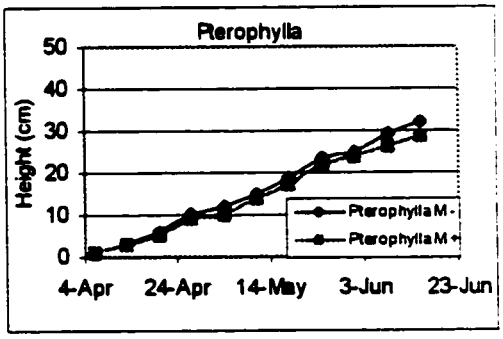

Trailblazer
Height (cm)
50
40
30
20
10
0
4-Apr
24-Apr
14-May
3-Jun
23-Jun
Trailblazer M-
Trailblazer M+

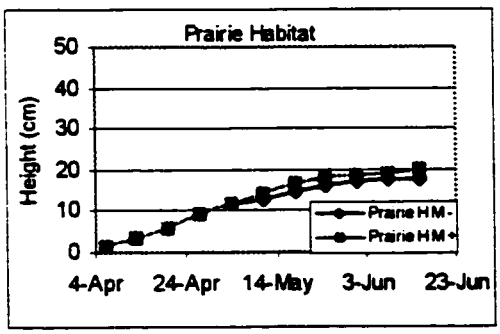

Caddo
Height (cm)
50
40
30
20
10
0
4-Apr
24-Apr
14-May
3-Jun
23-Jun
Caddo M-
Caddo M+

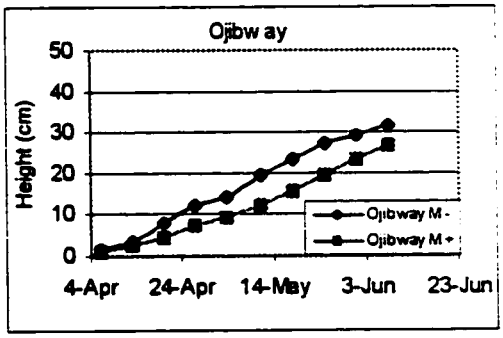

Summer
Height (cm)
50
40
30
20
10
0
4-Apr
24-Apr
14-May
3-Jun
23-Jun
Summer M-
Summer M+


Northern Upland
Height (cm)
50
40
30
20
10
0
4-Apr
24-Apr
14-May
3-Jun
23-Jun
Northern U M-
Northern U M+


ONP
Height (cm)
50
40
30
20
10
0
4-Apr
24-Apr
14-May
3-Jun
23-Jun
ONP M-
ONP M+


Pterophylla
Height (cm)
50
40
30
20
10
0
4-Apr
24-Apr
14-May
3-Jun
23-Jun
Pterophylla M-
Pterophylla M+


Prairie Habitat
Height (cm)
50
40
30
20
10
0
4-Apr
24-Apr
14-May
3-Jun
23-Jun
Prairie H M-
Prairie H M+


Ojibway
Height (cm)
50
40
30
20
10
0
4-Apr
24-Apr
14-May
3-Jun
23-Jun
Ojibway M-
Ojibway M+

3.0- Growth Rate

Growth rate was measured by the height difference of the first experiment plants. The cultivars grew to a greater height than the wild varieties (Figs. 3.1-3.10). The difference in plant height between cultivars and wild varieties was present from the germination stage. The growth curve profiles for mycorrhizal (M+) and non-mycorrhizal (M-) treatments were similar for most of the varieties. Trailblazer and Ojibway showed a divergence where the M- plants grew taller. These differences were significant at the time of harvest (see section 6.3). By the end of the 12-week growth period, most of the plants had reached a growth plateau.

4.0 - Chlorophyll Concentration

Overall, the chlorophyll concentration was quite similar among varieties and M+/M- treatments (Table 3.3). Variety ($p= 0.000$) and mycorrhizal treatment ($p=0.000$) were both significant effects, without significant interaction ($p= 0.913$). Caddo, Summer, NU and Trailblazer had lower chlorophyll levels than Pterophylla, Forestburg and Shelter. The M- treatment had a significantly higher average chlorophyll concentration (24.11) than the M+ treatment (22.93). Wild plants were found to have significantly higher (24.47) chlorophyll concentrations than cultivars (22.93) ($p= 0.040$), with no interaction between cultivation level and treatment ($p=0.873$).

Table 3.3. Chlorophyll concentration ± standard error in switchgrass varieties.

Cultivation Level	Variety	Chlorophyll Concentration		
		M-	M+	
Wild	Prairie Habitat	26.14 ±1.21	24.87 ±1.15	ab
	ONP	24.11 ±0.47	22.00 ±0.61	bc
	Ojibway	23.65 ±0.56	23.06 ±0.79	bc
	Pterophylla	26.92 ±0.94	24.98 ±1.04	a
Cultivated	Shelter	24.70 ±0.54	22.97 ±0.59	abc
	Forestburg	26.19 ±0.98	23.74 ±1.47	ab
	Summer	23.19 ±0.86	21.38 ±0.88	c
	Caddo	21.74 ±1.33	21.43 ±0.58	c
	Trailblazer	22.21 ±0.91	22.18 ±0.72	c
	Northern Upland	22.94 ±0.98	22.52 ±0.67	c
Wild		25.20	23.73	α
Cultivated		23.49	22.37	β

Significant differences between M-/+ treatments for all varieties determined by a two-way ANOVA at $\alpha = 0.05$ with no interaction between myc and variety indicated by ♦.
Significant differences between varieties as determined by a Tukey's test at $\alpha = 0.05$ indicated by different letters.
Significant differences between cultivation level as determined by a two-way ANOVA at $\alpha = 0.05$ indicated by α, β.

5.0 - Mycorrhizal Colonization

First Experiment

On average, 37% of the plant roots were colonized by *Glomus intraradices* (Fig 3.11). There was a significant effect of variety on colonization level ($p=0.007$), which was due to a difference between Caddo (59%) and ONP (21%) based on a Tukey's test. The cultivation level did not affect the percentage of mycorrhizal colonization. No mycorrhizae were observed in the non-mycorrhizal plants.

Second Experiment

The colonization percentage was similar in the second experiment, with the average being 36% (Fig 3.12). No statistical differences were detected among the varieties or between the cultivation levels. As in the first experiment, no mycorrhizae were observed in the non-mycorrhizal plants.

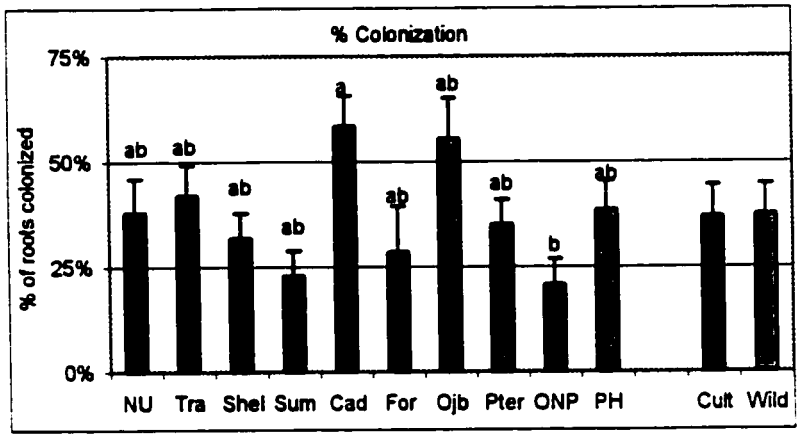


Figure 3.11. The colonization percentage with *G. intraradices* from the first experiment. Switchgrass varieties are indicated on the x-axis. Different letters indicate significant differences as detected by a Tukey's test at $\alpha = 0.05$.

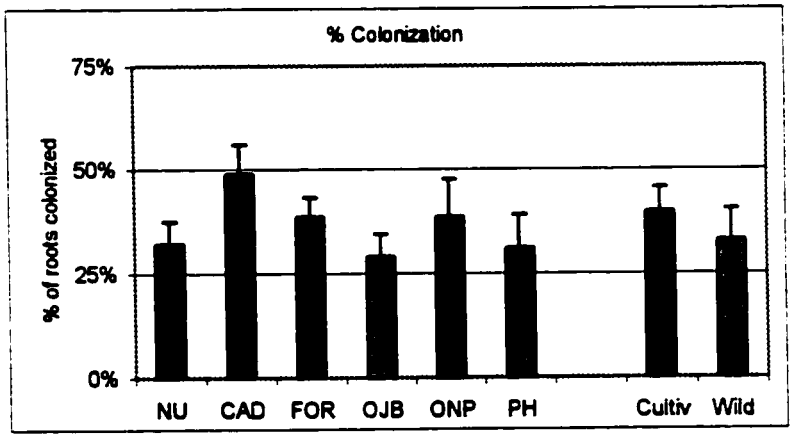


Figure 3.12. The colonization percentage with *G. intraradices* from the second experiment. Switchgrass varieties are indicated on the x-axis. No significant differences were detected.

6.0 - Physiological Data

6.1- Mass

First Experiment

The cultivars had significantly higher fresh and dry masses than wild varieties (Table 3.4). The mycorrhizal treatment decreased the fresh and dry masses for cultivars as a group, due to a decrease in the shoots rather than the roots (Fig. 3.13). Significant differences among varieties were found for fresh, dry, shoot and root masses (Table 3.4.1). Because of significant interactions, each variety was analyzed separately for mycorrhizal effect. Dry mass decreased significantly in the Ojibway, Pterophylla, Caddo, Trailblazer and NU varieties and increased in the ONP and Shelter varieties with the mycorrhizal treatment (Table 3.4). Fresh mass decreased significantly in PH, Ojibway, Pterophylla, Shelter, Caddo and Trailblazer and increased in Summer under the M+ treatment.

Second Experiment

As in the first experiment, cultivars had significantly higher fresh and dry masses than the wild varieties (Table 3.5). A significant varietal effect was found for fresh, dry, shoot and root masses (Tables 3.5 and 3.4.1). The mycorrhizal treatment effect was not significant and did not affect mass for most of the varieties (Table 3.4.1). Varieties were analyzed separately because of a significant interaction with

mycorrhizae. Significant increases in mass with the mycorrhizal treatment were seen in Forestburg for dry, root and shoot mass; NU for fresh and root masses; and ONP for fresh mass (Table 3.5). The increases in mass with the mycorrhizal treatment were not significant over cultivation levels (Fig. 3.17 and Table 3.4.1).

6.2 – Root to Shoot Ratio

First Experiment

The wild varieties had significantly higher root to shoot ratios (R/S) than the cultivated varieties (Table 3.4). Mycorrhizae significantly increased the R/S for both cultivation levels (Fig. 3.14). Variety and mycorrhizal treatment had significant effects and interaction (Table 3.4.1). The increased R/S with mycorrhizae was significant for ONP, Ojibway, Summer, and Caddo (Table 3.4). Among the varieties, PH and Forestburg had the highest R/S while ONP and Caddo had the lowest. Other varieties were intermediate.

Second Experiment

Varieties had significantly different R/S ratios (Table 3.5). The R/S of PH and Forestburg were found to be significantly higher than the others. There was no significant mycorrhizal treatment effect or interaction detected for the R/S (Table 3.4.1). The wild and cultivated groups were not different from each other (Fig. 3.19).

6.3 – Plant Height

First Experiment

Cultivated plants had a superior height compared to wild plants after the 12-week growth period (Table 3.4). Significant varietal, mycorrhizal and interaction effects were found for height (Table 3.4.1). PH plants were much smaller than the others; NU and Caddo grew to the greatest height (Table 3.4). For ONP and Forestburg the height increased with the M+ treatment, in Ojibway, Pterophylla, Shelter, Caddo, and Trailblazer the height decreased with mycorrhizae. Over both cultivation levels, the mycorrhizae decreased the total height of the plants (Fig. 3.15).

Second Experiment

The height of cultivated plants was greater than that of the wild varieties (Table 3.5; Fig. 3.19). A significant effect of variety on height was detected, while mycorrhizal effect and the interaction were non-significant (Table 3.4.1). PH was found to be shorter than all the rest; NU and Caddo were the tallest varieties (Table 3.5).

6.4 – Number of Tillers

First Experiment

The number of vegetative shoots was found to be higher in the wild varieties than the cultivars; this difference was greater in the M+ treatment (Table 3.4). A decrease in tiller numbers with mycorrhizae was found over both cultivation levels (Fig. 3.16). Variety, mycorrhizal treatment and their interaction all had significant effects (Table 3.4.1). PH, ONP, Caddo and Trailblazer had the largest amounts of tillers, while Shelter had the least (Table 3.4). Tiller numbers decreased significantly in M+ plants for Ojibway, Pterophylla, Summer, Caddo, Trailblazer, and NU.

Second Experiment

The cultivated and wild plants had similar tiller numbers in this experiment (Table 3.5). The decrease in tiller numbers with mycorrhizae for both wild and cultivated groups seen in the first experiment was also found in the second experiment (Fig 3.20). There was no significant effect of variety, but there was a significant mycorrhizal effect and an interaction term (Table 3.4.1). The tiller number decrease was significant in Caddo and NU (Table 3.5).

6.5- Summary of Physiological Parameters

First Experiment

Cultivated varieties had higher mass and height than the wild plants. However, root/ shoot ratio was higher in the wild plants than the cultivars. The shoot mass, height and tiller numbers all decreased with the mycorrhizal treatment as a general trend while R/S increased. There was a significant varietal effect for all the physiological factors. The effect of mycorrhizae was dependent upon variety because of significant interactions. Ojibway, Pterophylla, Caddo, and Trailblazer showed a consistent significant negative effect to the mycorrhizal treatment. ONP and Forestburg had positive responses to mycorrhizae. The varieties PH, Shelter, Summer and NU had no clear response to the mycorrhizal treatment.

Second Experiment

Varieties were quite widespread in their physiological parameters and general response to the mycorrhizal treatment. Cultivated plants had higher mass, height and than wild plants. Mycorrhizae tended to increase the mass but decrease the tiller numbers. Significant varietal differences were found for all the physiological parameters except tiller numbers. The mycorrhizal treatment did not have a significant effect in most cases. ONP and Forestburg responded positively to mycorrhizae, other varieties showed no clear response trend.

Table 3.4. Physiological data for experiment 1 switchgrass plants ± standard error.

	Variety	Fresh Mass (g)			Dry Mass (mg)			Root Mass (mg)			Shoot Mass (mg)		
		M-	M+		M-	M+		M-	M+		M-	M+	
Wild	Prairie Habitat	0.93 ±0.04 *	0.56 ±0.03	f	189 ±12	179 ±16	e	105 ±9	89 ±11	e	84 ±5	90 ±16	e
	ONP	1.20 ±0.04	1.28 ±0.03	bcd	248 ±11 *	340 ±25	cd	93 ±6 *	167 ±25	d	155 ±6	173 ±6	cd
	Ojibway	1.55 ±0.06 *	1.11 ±0.04	bc	312 ±16 *	286 ±8	bcd	134 ±8	133 ±5	cd	177 ±8 *	153 ±4	cd
	Pterophylla	1.31 ±0.04 *	1.07 ±0.02	de	322 ±8 *	266 ±7	d	146 ±5 *	126 ±4	cd	176 ±5 *	140 ±3	d
Cultivated	Shelter	1.26 ±0.04 *	0.93 ±0.03	e	275 ±22 *	283 ±8	c	144 ±7 *	131 ±4	bc	164 ±7	152 ±5	cd
	Forestburg	1.63 ±0.05	1.72 ±0.04	a	336 ±7	351 ±8	a	164 ±6 *	179 ±5	a	172 ±5	172 ±5	bcd
	Summer	1.13 ±0.02 *	1.32 ±0.03	cd	348 ±7	332 ±7	ab	150 ±5	156 ±5	b	198 ±5 *	176 ±5	ab
	Caddo	1.58 ±0.03 *	1.12 ±0.02	b	371 ±9 *	294 ±8	ab	130 ±4	121 ±4	c	241 ±7 *	173 ±5	a
	Trailblazer	1.45 ±0.07 *	1.03 ±0.05	bcd	342 ±23 *	261 ±16	bcd	148 ±9 *	109 ±7	cd	195 ±14 *	152 ±9	bc
	Northern Upland	1.29 ±0.03	1.33 ±0.02	bc	376 ±7 *	335 ±7	a	157 ±5	148 ±4	ab	219 ±5 *	188 ±4	a
Wild		1.25 *	1.00	α	268	268	α	120	129	α	148 *	139	α
Cultivated		1.39 *	1.24	β	341 *	309	β	149	141	β	198 *	169	β

Significant differences between M-/+ treatments determined by a t-test at α =0.05 indicated by *.
Significant differences between varieties as determined by a Tukey's test at α =0.05 indicated by different letters.
Significant differences between cultivation level as determined by a two-way ANOVA at α =0.05 indicated by α, β.

Table 3.4 continued. Physiological data for experiment 1 switchgrass plants ± standard error.

	Variety	R/S			Height (cm)			# Tillers		
		M-	M+		M-	M+		M-	M+	
Wild	Prairie Habitat	1.36 ±0.22	1.18 ±0.10	a	19.78 ±0.64	18.98 ±0.75	f	2.37 ±0.14	2.22 ±0.10	a
	ONP	0.59 ±0.03 *	1.05 ±0.22	de	34.26 ±0.59 *	36.40 ±0.62	c	2.22 ±0.11	2.24 ±0.12	a
	Ojibway	0.75 ±0.02 *	0.87 ±0.03	bc	34.35 ±0.53 *	31.69 ±0.39	de	2.33 ±0.08 *	1.96 ±0.09	abc
	Pterophylla	0.84 ±0.03	0.90 ±0.03	b	33.94 ±0.52 *	30.09 ±0.44	de	2.29 ±0.10 *	1.92 ±0.08	abc
Cultivated	Shelter	0.87 ±0.04	0.88 ±0.03	b	32.86 ±0.62 *	29.28 ±0.45	e	1.96 ±0.11	1.80 ±0.10	c
	Forestburg	0.98 ±0.04	1.06 ±0.04	a	32.90 ±0.55 *	35.29 ±0.61	cd	1.88 ±0.10	1.90 ±0.10	bc
	Summer	0.78 ±0.04 *	0.90 ±0.04	bc	35.45 ±0.38	35.70 ±0.44	c	2.29 ±0.11 *	1.71 ±0.10	abc
	Caddo	0.55 ±0.02 *	0.70 ±0.02	e	41.77 ±0.66 *	39.42 ±0.52	ab	2.49 ±0.08 *	2.00 ±0.10	a
	Trailblazer	0.81 ±0.06	0.71 ±0.03	cd	39.62 ±1.02 *	35.67 ±0.94	b	2.56 ±0.15 *	2.10 ±0.11	a
	Northern Upland	0.73 ±0.03	0.80 ±0.02	cd	41.63 ±0.65	41.80 ±0.52	a	2.53 ±0.10 *	1.90 ±0.10	ab
Wild		0.89 *	1.00	α	30.58 *	29.29	α	2.30 *	2.09	α
Cultivated		0.78 *	0.84	β	37.37 *	36.19	β	2.28 *	1.90	β

Significant differences between M-/+ treatments determined by a t-test at $\alpha = 0.05$ indicated by *.
Significant differences between varieties as determined by a Tukey's test at $\alpha = 0.05$ indicated by different letters.
Significant differences between cultivation level as determined by a two-way ANOVA at $\alpha = 0.05$ indicated by α, β.

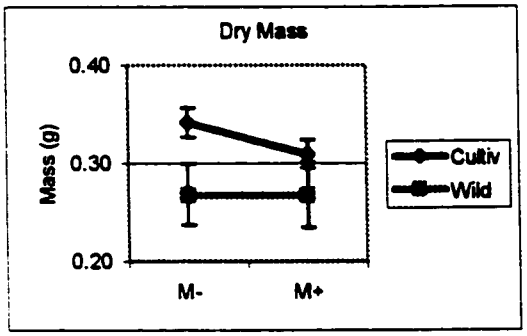


Fig 3.13. Dry mass

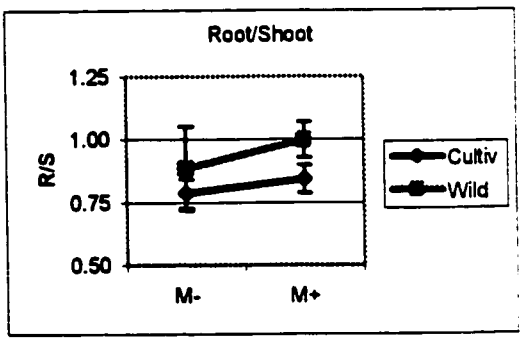


Fig 3.14. Root/Shoot Ratio

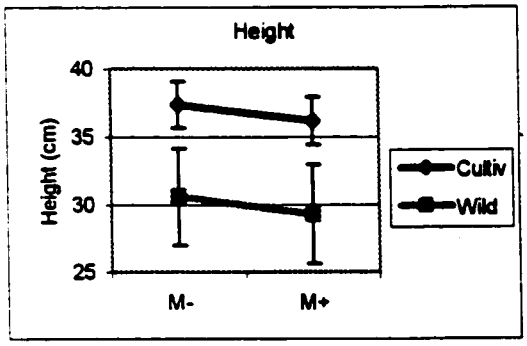


Fig 3.15. Plant Height

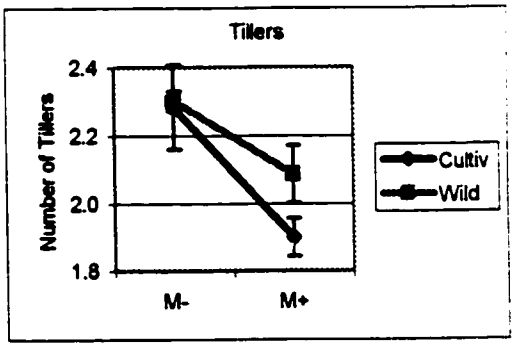


Fig 3.16. Number of Tillers

Figures 3.13-3.16. Physiological parameters of experiment 1 switchgrass plants. Differences between cultivation level and mycorrhizal treatment are shown for dry mass, R/S, height and tiller numbers. Standard error bars are indicated. Line graphs are used to visualize the interaction between cultivation level and mycorrhizal treatment; it is not intended to suggest any continuous variable.

Table 3.4.1. Results of two-way ANOVAs at $\alpha = 0.05$ for physiological parameters of the switchgrass plants from the first and second experiments. The first ANOVA was done for variety and mycorrhizal treatment, the second for cultivation level and mycorrhizal treatment.

	First ANOVA				Second ANOVA			
	R^2	V, p=	T, p=	V×T, p=	R^2	C, p=	T, p=	C×T, p=
Exp 1, n= 980								
Dry Mass*	0.437	0.000	0.000	0.000	0.172	0.000	0.004	0.005
R/S*	0.388	0.000	0.000	0.000	0.049	0.013	0.000	0.381
Height	0.714	0.000	0.000	0.000	0.216	0.000	0.005	0.804
Tillers*	0.102	0.000	0.000	0.000	0.050	0.026	0.000	0.091
Exp 2, n= 220								
Dry Mass*	0.572	0.000	0.270	0.007	0.308	0.000	0.229	0.897
R/S*	0.570	0.000	0.610	0.861	0.020	0.220	0.636	0.561
Height*	0.513	0.000	0.335	0.093	0.364	0.000	0.212	0.905
Tillers	0.236	0.508	0.000	0.000	0.089	0.905	0.000	0.038

Use of a non-parametric test indicated by *.
V= variety, T= mycorrhizal treatment, C= cultivation level.

Table 3.5. Physiological data for experiment 2 switchgrass plants ± standard error.

	Variety	Fresh Mass (g)			Dry Mass (mg)			Root Mass (mg)			Shoot Mass (mg)		
		M-	M+		M-	M+		M-	M+		M-	M+	
Wild	Prairie Habitat	1.56 ±0.13	1.94 ±0.14	d	376 ±37	357 ±36	c	169 ±17	161 ±14	b	207 ±27	196 ±25	c
	ONP	2.12 ±0.11 *	2.88 ±0.27	bc	461 ±17	615 ±80	b	133 ±11	161 ±25	b	328 ±10	454 ±59	b
	Ojibway	2.23 ±0.26	1.77 ±0.17	cd	336 ±58	286 ±14	c	92 ±22	82 ±8	c	244 ±40	204 ±69	c
Cultivated	Forestburg	2.93 ±0.11	3.47 ±0.24	a	635 ±52 *	972 ±61	a	232 ±18 *	340 ±15	a	403 ±35 *	632 ±51	a
	Caddo	2.92 ±0.23	2.69 ±0.16	ab	604 ±45	505 ±34	b	181 ±19	139 ±13	b	423 ±31	366 ±26	ab
	Northern Upland	2.89 ±0.13 *	2.25 ±0.20	b	563 ±35	619 ±53	b	154 ±9 *	165 ±16	b	409 ±28	454 ±37	ab
Wild		1.97	2.20	α	391	419	α	131	134	α	260	285	α
Cultivated		2.92	2.80	β	601	699	β	189	215	β	412	484	β

Significant differences between M-/+ treatments determined by a t-test at α =0.05 indicated by *.
Significant differences between varieties as determined by a Tukey's test at α =0.05 indicated by different letters.
Significant differences between cultivation level as determined by a two-way ANOVA at α =0.05 indicated by α, β.

Table 3.5 continued. Physiological data for experiment 2 switchgrass plants ± standard error.

	Variety	R/S			Height (cm)			# Tillers		
		M-	M+		M-	M+		M-	M+	
Wild	Prairie Habitat	0.90 ±0.12	0.91 ±0.11	a	30.78 ± 1.99	40.69 ±2.75	c	2.3 ±0.14	1.9 ±0.18	a
	ONP	0.41 ±0.03	0.36 ±0.04	b	50.57 ± 1.70	49.31 ±2.07	b	1.9 ±0.19	2.0 ±0.20	a
	Ojibway	0.36 ±0.07	0.42 ±0.07	b	49.98 ±3.51	47.46 ±2.09	b	2.1 ±0.31	1.6 ±0.27	a
Cultivated	Forestburg	0.58 ±0.02	0.56 ±0.05	a	54.98 ± 1.71	53.65 ±2.30	b	1.6 ±0.13	2.1 ±0.21	a
	Caddo	0.43 ±0.03	0.39 ±0.04	b	58.69 ± 1.45	63.02 ± 1.43	a	2.5 ±0.11 *	1.5 ±0.14	a
	Northern Upland	0.38 ±0.02	0.36 ±0.01	b	60.62 ± 1.58	64.30 ±2.39	a	2.9 ±0.21 *	1.4 ±0.13	a
Wild		0.56	0.56	α	43.78	45.82	α	2.1 *	1.8	α
Cultivated		0.47	0.44	α	58.10	60.32	β	2.3 *	1.6	α

Significant differences between M-/+ treatments determined by a t-test at α =0.05 indicated by *.
Significant differences between varieties as determined by a Tukey's test at α =0.05 indicated by different letters.
Significant differences between cultivation level as determined by a two-way ANOVA at α =0.05 indicated by α, β.

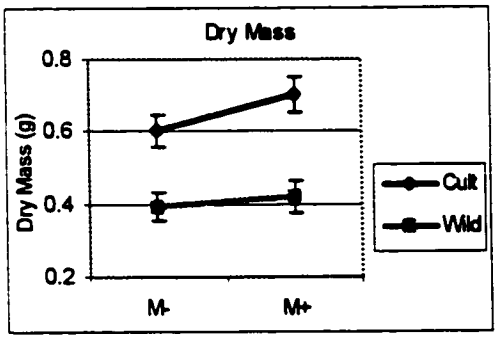


Fig 3.17. Dry Mass

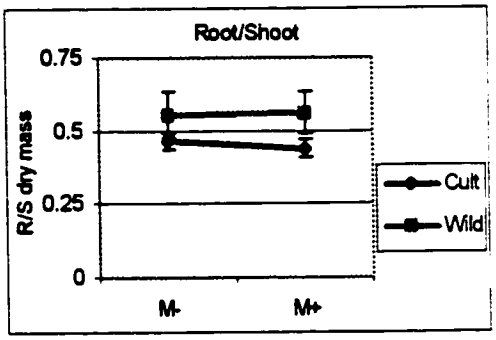


Fig 3.18. Root /Shoot ratio

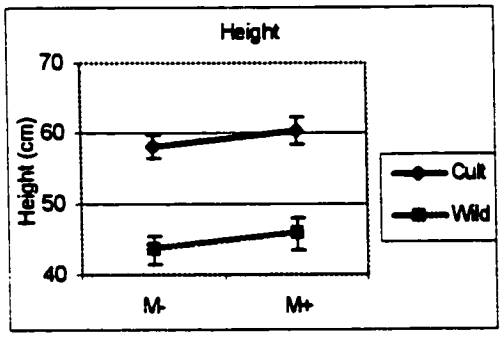


Fig 3.19. Plant Height

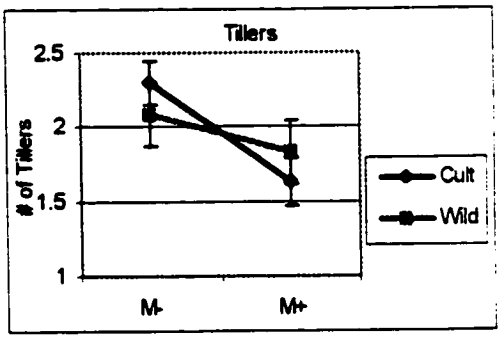


Fig 3.20. Number of Tillers

Figures 3.17-3.20. Physiological parameters of switchgrass from experiment 2 plants. Differences between cultivation level and mycorrhizal treatment are shown for dry mass, R/S, height and tiller numbers. Standard error bars are indicated. Line graphs are used to visualize the interaction between cultivation level and mycorrhizal treatment; it is not intended to suggest any continuous variable.

7.0 – Acid Phosphatase

First Experiment

Cultivated plants had significantly (p=0.012) lower phosphatase levels in the roots than wild plants (Table 3.6). The acid phosphatase levels decreased significantly in the mycorrhizal treatment for the cultivated group only (p=0.000) (Fig. 3.21). Significant differences were found for variety, mycorrhizal treatment and their interaction (p=0.000,0.000,0.000). The highest levels of acid phosphatase were found in the varieties PH, ONP, Ojibway, Shelter, and Trailblazer (Table 3.6). The acid phosphatase level decreased significantly in mycorrhizal Shelter, Forestburg, Trailblazer and NU.

Second Experiment

Cultivars had significantly (p=0.010) lower acid phosphatase levels than wild varieties in the second experiment as well (Table 3.7; Fig. 3.22). The mycorrhizal treatment did not have a significant effect (p=0.614) on the acid phosphatase level in any of the varieties. Differences between varieties were detected (p=0.000) but the interaction with mycorrhizae was not significant (p=0.170). The highest acid phosphatase levels were found in PH and Caddo.

Table 3.6. Acid phosphatase levels ± standard error for experiment 1 switchgrass plants expressed in ΔOptical Density at 400 nm/g FM roots/hr.

	Variety	ΔO.D 400 nm/g/hr		
		M-	M+	
Wild	Prairie Habitat	2.14 ± 0.20	2.57 ± 0.22	a
	ONP	2.17 ± 0.14	1.69 ± 0.13	a
	Ojibway	1.70 ± 0.16	2.46 ± 0.28	a
	Pterophylla	1.34 ± 0.09	1.12 ± 0.06	c
Cultivated	Shelter	2.76 ± 0.18	1.83 ± 0.09 *	a
	Forestburg	2.16 ± 0.20	1.12 ± 0.11 *	b
	Summer	1.47 ± 0.12	1.18 ± 0.10	bc
	Caddo	1.29 ± 0.16	1.29 ± 0.08	bc
	Trailblazer	2.36 ± 0.15	1.57 ± 0.11 *	a
	Northern Upland	1.89 ± 0.12	1.04 ± 0.06 *	bc
Wild		1.84 ± 0.02	1.96 ± 0.05	α
Cultivated		2.30 ± 0.01	1.51 ± 0.01 *	β

Significant differences between M-/+ treatments determined by a t-test at α =0.05 indicated by *.
Significant differences between varieties as determined by a Tukey's test at α =0.05 indicated by different letters.
Significant differences between cultivation level as determined by a two-way ANOVA at α =0.05 indicated by α, β.

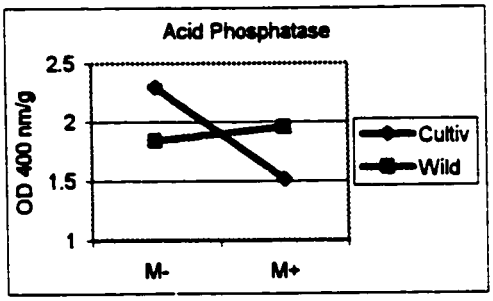


Fig 3.21. Acid phosphatase levels, experiment 1 switchgrass plants

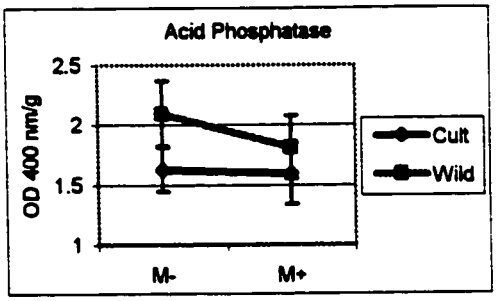


Fig. 3.22. Acid phosphatase levels, experiment 2 switchgrass plants

Figures 3.21-3.22. Acid phosphatase levels of switchgrass expressed in ΔOptical Density at 400 nm/g FM roots/hr. Comparisons of cultivation level and mycorrhizal treatment are shown for both experiments. Standard errors bars are shown. Line graphs are used to visualize the interaction between cultivation level and mycorrhizal treatment; it is not intended to suggest any continuous variable.

Table 3.7. Acid phosphatase levels ± standard error for experiment 2 switchgrass plants expressed in ΔOptical Density at 400 nm/g FM roots/hr.

	Variety	ΔO.D 400nm/g/hr		
		M-	M+	
Wild	Prairie Habitat	2.59 ± 0.34	1.98 ± 0.19	a
	ONP	1.85 ± 0.18	1.52 ± 0.24	ab
	Ojibway	1.83 ± 0.30	1.94 ± 0.36	ab
Cultivated	Forestburg	1.06 ± 0.07	1.41 ± 0.16	b
	Caddo	2.65 ± 0.43	1.86 ± 0.23	a
	Northern Upland	1.17 ± 0.13	1.49 ± 0.15	b
Wild		2.09± 0.27	1.81 ± 0.26	α
Cultivated		1.63 ± 0.18	1.59 ± 0.25	β

Significant differences between varieties as determined by a Tukey's test at α =0.05 indicated by different letters.
Significant differences between cultivation level as determined by a two-way ANOVA at α =0.05 indicated by α, β.

8.0 - Root Architecture

The root parameters most relevant to this study were total root length, average root diameter, number of root tips (to represent branching), the length of roots with small diameter (0-0.1mm) and the length of roots with large diameter (0.9 mm and over).

8.1 – Total Root Length

First Experiment

The cultivated and wild groups did not differ in their root lengths (Table 3.8). Significant mycorrhizal and varietal effects were found, with no interaction (Table 3.8.1). The mycorrhizal treatment significantly increased total root length for both the wild and cultivated groups (Fig 3.23) and over all varieties. The longest root lengths were in Pterophylla and Trailblazer, while NU and PH had the shortest root lengths.

Second Experiment

Mycorrhizae did not have a significant effect on root length in the second experiment, though the M+ plants tended to have longer root lengths than M- plants (Fig. 3.28). Significant differences among varieties and a marginally significant interaction with mycorrhizae were detected (Table 3.8.1). Forestburg had the longest root lengths while Ojibway had the shortest (Table 3.9). Cultivation level did not have a

significant effect on root length. The overall root lengths were higher in the second experiment than in the first because of the increased soil volume available.

8.2 – Average Root Diameter

First Experiment

Root diameter varied significantly amongst varieties (Table 3.8). The varieties Shelter and Trailblazer had the largest diameters while PH had the smallest. Significant effects of variety, mycorrhizal treatment and a marginally significant interaction were detected (Table 3.8.1). There was a significant decrease in diameter with the mycorrhizal treatment overall and for Shelter, Summer, and NU (Table 3.8). Cultivars had a larger diameter than wild varieties in the M- treatment (Fig. 3.24). The mycorrhizal treatment effect was no longer significant when assessed over cultivation level (Table 3.8.1).

Second Experiment

Variety was the most important factor determining root diameter in the second experiment (Table 3.9). A significant effect of variety, and an interaction with mycorrhizal treatment was detected (Table 3.8.1). Forestburg, Caddo and PH had the largest root diameters while Ojibway had the smallest (Table 3.9). Mycorrhizae significantly increased root diameter for Forestburg and NU, and significantly decreased it for Ojibway. A root diameter increase with mycorrhizae was found to be

significant for the cultivars but not the wild varieties (Fig. 3.29). As in the first experiment, cultivars had overall a significantly larger root diameter than wild varieties in the second experiment.

8.3 – Number of Root Tips

First Experiment

Cultivation level did not affect the number of root tips, but mycorrhizae significantly increased root tip numbers for the wild varieties (Table 3.8; Fig. 3.25). The number of root tips was affected by significant effects of variety, mycorrhizal treatment and their interaction (Table 3.8.1). The greatest number of root tips was found in Pterophylla, the least in PH (Table 3.8). This result may be expected since Pterophylla has the largest root system and PH the smallest. The addition of mycorrhizae tended to increase root tip numbers. This increase was significant in Pterophylla, PH, and Caddo; a significant decrease occurred in NU.

Second Experiment

Root tip numbers tended to decrease in the M+ plants in this experiment (Fig. 3.30). No significant effects were found although variety was a marginally significant factor (Table 3.8.1). ONP had the greatest number of root tips (Table 3.9). The large standard error associated with this parameter might have contributed to the lack of significant differences.

8.4 – Percentage of Small Roots

Small roots include the length of root in the category of the smallest root diameter (< 0.1 mm). To make this length relative, it was divided by the total root length for a specific plant and multiplied by 100%. Of the total root length, 7.5% to 16% fell into this category in the first experiment, and 11 % to 16% in the second experiment.

First Experiment

Significant effects of variety, mycorrhizal treatment and their interaction were found for percentage of small root length (Table 3.8.1). Shelter and NU had the highest % small roots PH had the lowest (Table 3.8). Mycorrhizae significantly increased % small root length overall. Ojibway, Shelter, experienced significant increases with mycorrhizal treatment and NU experienced a decrease. Wild varieties had a significant increase with mycorrhizae but cultivars did not (Fig. 3.26). Cultivars had an overall larger % small root length.

Second Experiment

Varietal differences in small roots was less pronounced in the second experiment, but still statistically significant (Tables 3.9 and 3.8.1). ONP, Forestburg and NU had the highest % of small roots while PH had the lowest (Table 3.9). Mycorrhizae had the expected effect of decreasing % of small root length in the second experiment. This

effect was not dependent upon variety (Table 3.8.1). As in the first experiment, cultivars had significantly higher % small roots than wild varieties (Fig. 3.31).

8.5 – Percentage of Large Roots

Large roots include the length of root in the category of the largest root diameter ($\geq$ 0.9 mm). To make this length relative, it was divided by the total root length for a specific plant and multiplied by 100%. The percentage of roots that fell into this category was much less than the % of small roots. Percent of large roots were found to be between 1.0% - 3.2% in the first experiment, and 0.4% - 1.2% in the second.

First Experiment

Significant effects of variety, mycorrhizal treatment and their interaction were found for the length of large roots (Table 3.8.1). Trailblazer had the most % large roots while PH and ONP had the least (Table 3.8). Forestburg, Caddo and NU experienced a significant increase in % of large root length with mycorrhizae. The other varieties tended to increase % large roots in the M+ treatment, except for ONP and Shelter. Mycorrhizae significantly increased % large roots for cultivars. Cultivars had a higher % of large roots than wild varieties (Fig. 3.27).

Second Experiment

The percentage of large roots was much smaller in the second experiment (Table 3.9). A significant varietal effect (Table 3.8.1) was due to NU having a higher proportion of large roots than the others (Table 3.9). Mycorrhizae had no significant overall effect, but some varieties were affected. Ojibway had a significant decrease in the % large roots and Forestburg had a significant increase. No significant differences were found between the cultivation levels, although cultivars tended to increase their % large roots with M+ while wild varieties decreased theirs (Fig. 3.32).

6.6- Summary of Root Parameters

First Experiment

In general, mycorrhizae had the effect of increasing root length, the amount of root tips, and % small roots while decreasing root diameter. For % large roots, no definite trend was observed. Varietal differences existed for all the root parameters. Pterophylla, Shelter and Trailblazer had large root systems; PH had a small root system. In root tips and % small roots, where interactions with cultivation level and treatment occurred, wild varieties responded more dramatically to mycorrhizae.

Second Experiment

Fewer differences were detected in this experiment. For root length, number of root tips and % large roots, no definite trend was observed. Average root diameter decreased with mycorrhizae in wild varieties and significantly increased in cultivars; this was the only significant cultivation level interaction. An overall decrease in the % small roots was observed with mycorrhizae. Varietal differences existed for all root parameters except root tips. Forestburg had the largest root system; Ojibway's was smaller and had finer roots.

Table 3.8. Root architecture data ± standard error from experiment 1 switchgrass plants.

	Variety	Root Length (cm)			Average Root Diameter (mm)			Number of Root Tips		
		M-	M+		M-	M+		M-	M+	
Wild	Prairie Habitat	463.9 ±45.6 *	712.8 ±70.9	cd	0.342 ±0.009	0.350 ±0.007	c	919 ±108 *	1531 ±160	c
	ONP	615.1 ±96.3	907.7 ±128.2	bcd	0.647 ±0.055	0.564 ±0.053	ab	1351 ±224	2223 ±343	bc
	Ojibway	704.0 ±71.6	792.0 ±99.9	bcd	0.436 ±0.050	0.427 ±0.061	bc	1532 ±219	1719 ±266	abc
	Pterophylla	1041.6 ±95.7	1176.5 ±103.0	a	0.583 ±0.058	0.647 ±0.058	ab	2122 ±220 *	3107 ±350	a
Cultivated	Shelter	784.4 ±40.5	895.0 ±81.9	bc	0.760 ±0.023 *	0.520 ±0.059	a	2141 ±94	2366 ±191	ab
	Forestburg	750.0 ±74.6	868.5 ±99.2	bc	0.647 ±0.046	0.521 ±0.067	ab	1598 ±183	1670 ±134	abc
	Summer	595.6 ±49.2 *	743.9 ±30.9	bcd	0.452 ±0.046 *	0.377 ±0.009	bc	1475 ±169	1782 ±200	abc
	Caddo	598.5 ±35.5 *	868.1 ±112.8	bcd	0.380 ±0.006	0.522 ±0.074	b	1185 ±118 *	1949 ±315	bc
	Trailblazer	892.0 ±59.5	939.2 ±69.1	ab	0.750 ±0.022	0.603 ±0.076	a	1664 ±161	1827 ±138	abc
	Northern Upland	524.8 ±61.1	568.7 ±42.0	d	0.626 ±0.065 *	0.412 ±0.013	ab	1991 ±227 *	1096 ±98	bc
Wild		706.1 ♦	897.2	α	0.505	0.497	α	1481 *	2145	α
Cultivated		690.9 ♦	813.9	α	0.603	0.493	β	1676	1782	α

Significant differences between M-/+ treatments for all varieties determined by a two-way ANOVA at $\alpha = 0.05$ with no interaction between myc and variety indicated by ♦.
Significant differences between M-/+ treatments determined by a t-test at $\alpha = 0.05$ indicated by *.
Significant differences between varieties as determined by a Tukey's test at $\alpha = 0.05$ indicated by different letters. Significant differences between cultivation level as determined by a two-way ANOVA at $\alpha = 0.05$ indicated by α, β.

Table 3.8 continued. Root architecture data from experiment 1 switchgrass plants ± standard error.

	Variety	% Small Root Length			% Large Root Length		
		M-	M+		M-	M+	
Wild	Prairie Habitat	7.5 ±1.33	9.2 ±1.68	c	0.8 ±0.16	0.8 ±0.12	c
	ONP	9.8 ±1.94	11.7 ±2.31	bc	1.3 ±0.22	1.0 ±0.23	c
	Ojibway	8.9 ±1.68	12.2 ±2.12 *	bc	1.5 ±0.32	2.5 ±0.70 *	ab
	Pterophylla	9.8 ±1.48	12.5 ±1.55	abc	1.1 ±0.25	1.7 ±0.25	bc
Cultivated	Shelter	10.1 ±0.60	13.8 ±1.39 *	a	3.0 ±0.40	2.0 ±0.15	ab
	Forestburg	8.6 ±1.36	9.6 ±1.12	bc	1.0 ±0.09	2.1 ±0.29 *	bc
	Summer	11.4 ±1.74	12.9 ±1.34	ab	1.8 ±0.22	2.2 ±0.29	ab
	Caddo	9.5 ±1.44	11 ±1.71	bc	1.5 ±0.23	2.7 ±0.45 *	ab
	Trailblazer	8.9 ±1.3	9.5 ±0.59	bc	2.5 ±0.22	2.8 ±0.14	a
	Northern Upland	16 ±2.14	9.8 ±1.41 *	a	1.6 ±0.57	3.2 ±0.37 *	ab
Wild		9.0	11.4 *	α	1.2	1.5 *	α
Cultivated		10.7	11.1	β	1.9	2.5 *	β

Significant differences between M-/+ treatments determined by a t-test at $\alpha = 0.05$ indicated by *.
Significant differences between varieties as determined by a Tukey's test at $\alpha = 0.05$ indicated by different letters.
Significant differences between cultivation level as determined by a two-way ANOVA at $\alpha = 0.05$ indicated by α, β.

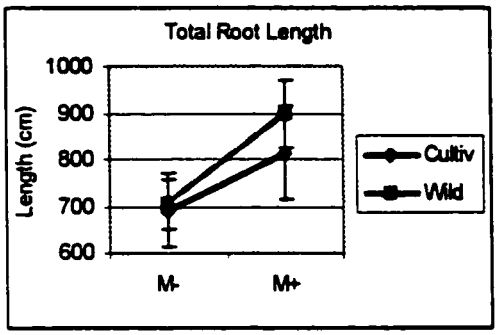


Fig 3.23. Total root length

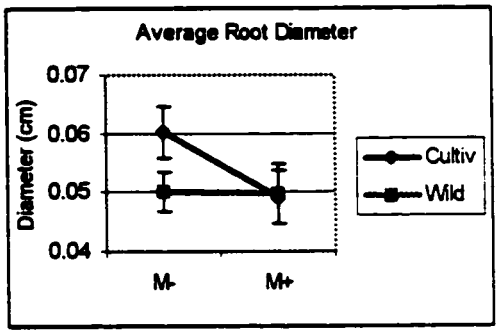


Fig 3.24. Average root diameter

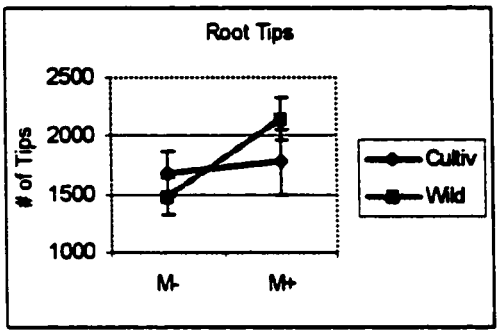


Fig 3.25. Number of root tips

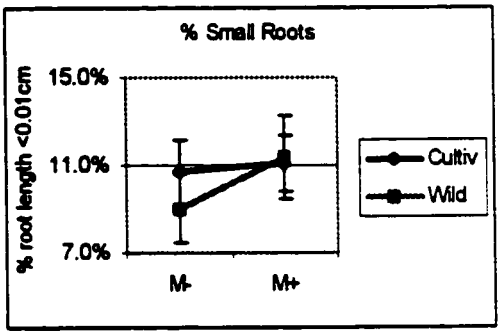


Fig 3.26. % Small roots

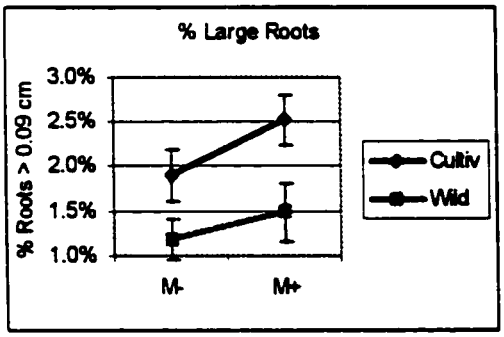


Fig 3.27. % Large roots

Figures 3.23-3.27. Root architecture parameters of switchgrass for experiment 1 plants. Differences between cultivation level and mycorrhizal treatment are shown for root length, root diameter, number of root tips, % small and large roots. Standard error bars are shown. Line graphs are used to visualize the interaction between cultivation level and mycorrhizal treatment; it is not intended to suggest any continuous variable.

Table 3.8.1. Results of two way ANOVAs at $\alpha = 0.05$ for switchgrass root parameters. First ANOVA was done for variety and mycorrhizal treatment, second results are for cultivation level and mycorrhizal treatment.

	First ANOVA				Second ANOVA			
	R^2	V, p=	T, p=	V×T, p=	R^2	C, p=	T, p=	C×T, p=
Exp 1, n=140								
Root Length	0.458	0.000	0.000	0.731	0.095	0.224	0.000	0.442
Root Diameter*	0.500	0.000	0.005	0.060	0.106	0.003	0.055	0.174
Root Tips	0.466	0.000	0.001	0.001	0.088	0.812	0.002	0.018
% Small Roots	0.603	0.000	0.025	0.000	0.090	0.045	0.015	0.036
% Large Roots*	0.551	0.000	0.000	0.000	0.293	0.000	0.007	0.482
Exp 2, n=110								
Root Length	0.290	0.001	0.060	0.054	0.043	0.412	0.068	0.534
Root Diameter	0.429	0.000	0.128	0.000	0.198	0.017	0.075	0.000
Root Tips	0.153	0.088	0.119	0.367	0.028	0.565	0.165	0.464
% Small Roots	0.322	0.000	0.000	0.189	0.160	0.014	0.001	0.200
% Large Roots	0.367	0.000	0.567	0.001	0.057	0.435	0.920	0.190

Use of a Non-Parametric test indicated by *.
V= variety, T= mycorrhizal treatment, C= cultivation level.

Table 3.9. Root architecture data from experiment 2 switchgrass plants ± standard error.

	Variety	Root Length (cm)			Average Root Diameter (mm)			Number of Root Tips		
		M-	M+		M-	M+		M-	M+	
Wild	Prairie Habitat	974.1 ±101.1	1032.1 ±97.4	ab	0.359 ±0.007	0.359 ±0.003	a	2037 ±205	1961 ±515	a
	ONP	987.0 ±99.5	1094.1 ±65.5	ab	0.356 ±0.036	0.348 ±0.008	ab	2760 ±347	2788 ±237	a
	Ojibway	818.7 ±52.33	827.7 ±40.9	b	0.347 ±0.008	0.313 ±0.002 *	b	2407 ±146	1933 ±275	a
Cultivated	Forestburg	1011.3 ±51.0	1446.4 ±122.8	a	0.347 ±0.004	0.431 ±0.011 *	a	2527 ±126	2698 ±428	a
	Caddo	999.1 ±124.4	912.9 ±65.2	b	0.357 ±0.006	0.361 ±0.007	a	2378 ±291	2230 ±251	a
	Northern Upland	870.4 ±69.7	930.2 ±82.3	b	0.336 ±0.006	0.370 ±0.007 *	ab	2821 ±165	1850 ±162	a
Wild		926.4	984.6	α	0.353	0.340	α	2401	2227	α
Cultivated		960.3	1096.5	α	0.347	0.387 *	β	2575	2259	α

Significant differences between M-/+ treatments for all varieties determined by a two-way ANOVA at $\alpha = 0.05$ with no interaction between myc and variety indicated by ♦.
Significant differences between M-/+ treatments determined by a t-test at $\alpha = 0.05$ indicated by *.
Significant differences between varieties as determined by a Tukey's test at $\alpha = 0.05$ indicated by different letters.
Significant differences between cultivation level as determined by a two-way ANOVA at $\alpha = 0.05$ indicated by α, β.

Table 3.9 continued. Root architecture data from experiment 2 switchgrass plants ± standard error.

	Variety	% Small Root Length			% Large Root Length		
		M-	M+		M-	M+	
Wild	Prairie Habitat	13 ±0.90	11 ±0.74	b	0.7 $\pm 7\times10^{-4}$	0.8 $\pm 8\times10^{-4}$	ab
	ONP	14 ±1.37	15 ±0.71	a	0.9 $\pm 8\times10^{-4}$	0.8 $\pm 7\times10^{-4}$	a
	Ojibway	14 ±1.61	12 ±0.36	ab	1.1 $\pm 78\times10^{-4}$ *	0.4 $\pm 93\times10^{-4}$	ab
Cultivated	Forestburg	15 ±0.56	14 ±2.14	a	0.8 $\pm 9\times10^{-4}$ *	1.2 $\pm 7\times10^{-4}$	a
	Caddo	14 ±1.33	13 ±0.73	ab	0.9 $\pm 7\times10^{-4}$	0.9 $\pm 10\times10^{-4}$	a
	Northern Upland	16 ±0.8	13 ±1.0	a	0.6 $\pm 4\times10^{-4}$	1.1 $\pm 10\times10^{-4}$	b
		♦					
Wild		14	12	α	0.9	0.7	α
Cultivated		15	13	β	0.8	1.1	α
		♦					

Significant differences between M-/+ treatments for all varieties determined by a two-way ANOVA at $\alpha = 0.05$ with no interaction between myc and variety indicated by ♦.
Significant differences between M-/+ treatments determined by a t-test at $\alpha = 0.05$ indicated by *.
Significant differences between varieties as determined by a Tukey's test at $\alpha = 0.05$ indicated by different letters.
Significant differences between cultivation level as determined by a two-way ANOVA at $\alpha = 0.05$ indicated by α, β.

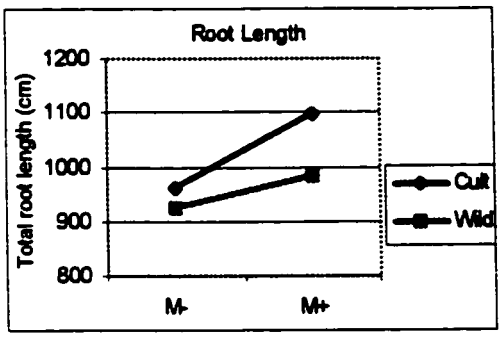


Fig 3.28. Total root length

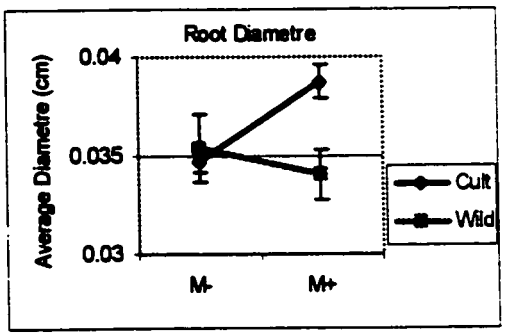


Fig 3.29. Average root diameter

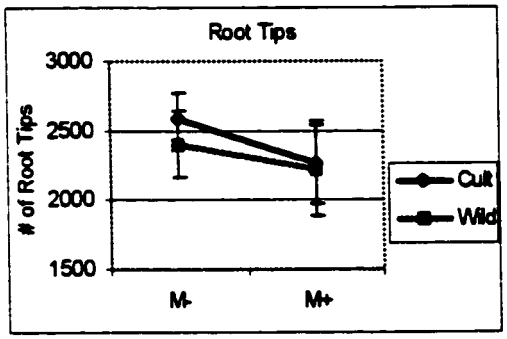


Fig 3.30. Number of root tips

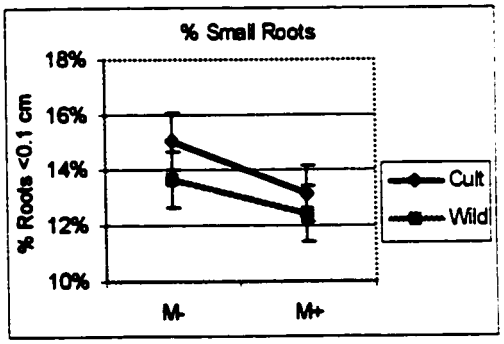


Fig 3.31. % Small roots

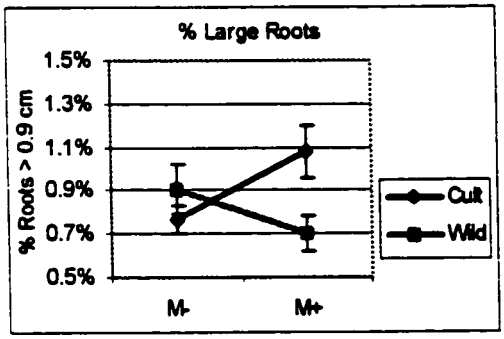


Fig 3.32. % Large roots

Figures 3.28-3.32. Root architecture parameters of switchgrass for experiment 2 plants. Differences between cultivation level and mycorrhizal treatment are shown for root length, root diameter, number of root tips, % small and large roots. Standard error bars are shown. Line graphs are used to visualize the interaction between cultivation level and mycorrhizal treatment, it is not intended to suggest any continuous variable.

9.0- Mineral Content

9.1 – Carbon

First Experiment

Roots and shoots contained similar carbon contents (%C), 45% and 44% respectively (Table 3.10). Significant effects of variety and mycorrhizal treatment were found for both roots and shoots; their interaction was significant in roots only (Table 3.11.1). A significant decrease in %C occurred in the mycorrhizal plants, even though the difference between M+ and M- was less than 1% in all cases, except in Forestburg roots (Table 3.10). Varieties differed by similarly small amounts. Carbon levels of cultivars were slightly higher than those of wild varieties; this difference was statistically significant (Figs. 3.33 and 3.34).

Second Experiment

As in the first experiment, %C was similar in roots (45%) and shoots (43%) (Table 3.11). In the shoots, a significant effect of variety and an interaction with the mycorrhizal treatment were found; in the roots, all factors were significant (Table 3.11.1). Caddo and Ojibway had a higher %C in shoots than ONP or PH. In roots, no differences among varieties were found despite the significant varietal effect. Mycorrhizae significantly increased %C in the shoots of Caddo. Wild varieties and cultivars were not significantly different from each other (Table 3.11) but %C of

roots significantly increased in wild varieties and decreased in cultivars (Figs. 3.37 and 3.38).

9.2 – Nitrogen

First Experiment

The total nitrogen content (%N) was higher in shoots (1.2%) than roots (0.8%) (Table 3.10). Significant effects of variety, mycorrhizal treatment and interaction were found for shoot %N; in roots all except the mycorrhizal effect were significant (Table 3.11.1). PH had high %N in both portions, while Caddo and Pterophylla had low %N (Table 3.10). Decreases in % N were noted in the shoots of mycorrhizal PH, Pterophylla, and Shelter. No mycorrhizal effect was found over cultivation levels (Table 3.11.1). Wild varieties had higher %N than cultivars, and these tended to decrease with the M+ treatment (Figs. 3.35 and 3.36).

Second Experiment

Similarly to the first experiment, shoots had a greater % N (1.4%) than roots (0.9%) (Table 3.11). Total N contents tended to be higher in the second experiment than the first. Significant effects were found for variety, mycorrhizal treatment and interaction in both shoots and roots (Table 3.11.1). In the shoots, PH had the highest %N, Forestburg and NU the lowest (Table 3.11). In the roots, Ojibway had the highest %N and Forestburg the lowest. Mycorrhizae significantly increased the %N in the shoots

of Caddo, and the roots of ONP, Caddo and Ojibway. The wild varieties had higher %N than the cultivars in both shoots and roots; significant increases in %N with M+ were seen in both cultivation levels (Figs. 3.39 and 3.40).

9.3- Phosphorus

Shoots contained higher P levels (970 μg/g) than roots (680 μg/g). The varietal effect and interaction were significant for roots and shoots; the mycorrhizal effect was significant for roots only (Table 3.11.2). The highest P concentrations were found in Ojibway (shoots) and PH (roots), and the lowest in Caddo (Table 3.12). Mycorrhizae significantly increased root P levels overall, and in Forestburg. Root P level significantly decreased with mycorrhizae in PH. The mycorrhizal effect was not significant over cultivation level groups (Table 3.11.2). The P concentration was significantly higher in wild varieties than cultivars (Figs. 3.41 and 3.42).

9.4- Calcium

Average calcium concentration was higher in shoots (1.5 mg/g) than roots (1.1 mg/g). A significant effect of variety was found for both roots and shoots while the interaction was significant for shoots (Table 3.11.2). No effects of the mycorrhizal treatment were found. Interestingly, PH had the highest Ca concentration in shoots and lowest in roots, while Caddo had the lowest Ca in shoots and highest in roots (Table 3.12). Wild varieties had higher shoot Ca concentrations than cultivars, no differences were found in roots (Figs. 3.43 and 3.44).

9.5- Potassium

Potassium levels were higher in the roots (21.8 mg/g) than the shoots (19.2 mg/g). Significant varietal effects and interactions are found in both roots and shoots; mycorrhizal treatment effect was significant in shoots only (Table 3.11.2). Varietal differences are due to PH (shoots and roots) and Forestburg (roots) having lower K levels than the others (Table 3.13). Increases with M+ were found in the shoots of ONP and the roots of Caddo, while a significant decrease was found in the root K level of Caddo. No differences were found between cultivars and wild varieties in either roots or shoots (Figs. 3.45 and 3.46).

9.6- Magnesium

Root sections contained higher Mg levels (4.0 mg/g) than shoots (2.2 mg/g) (Table 3.13). No significant effects were detected for variety, mycorrhizal treatment or interactions (Table 3.11.2). Cultivars and wild varieties had similar Mg concentrations in both roots and shoots (Figs. 3.47 and 3.48).

9.7 – Overall Mineral Effects

First Experiment

An increase in mineral content with mycorrhizae was not observed in the first experiment plants, when available soil volume was low. The N content tended to decrease in mycorrhizal plants, though this was not significant. Mycorrhizal plants had a significantly lower %C than the non-mycorrhizal ones, with the absolute difference between M- and M+ being very small in each case.

Second Experiment

Mineral levels were all in the expected range, except for high Mg. Roots contained on average, higher levels of N, K and Mg while shoots contained higher P and Ca levels. Mycorrhizae increased the concentrations of N, P, K and Ca depending on variety. Wild plants tended to respond positively to mycorrhizae with increased Ca concentrations while cultivars had higher P levels than wild types in the mycorrhizal treatment. Wild plants tended to have higher mineral concentrations than cultivated plants. No differences in %C were observed with the mycorrhizal treatment.

Table 3.10. Total percentage of C and N ± standard error for experiment 1 switchgrass plants.

	Variety	% C Shoot			% C Root			% N Shoot			%N Root		
		M-	M+		M-	M+		M-	M+		M-	M+	
Wild	Prairie Habitat	43.61 ±0.21	43.54 ±0.06	b	45.52 ±0.12	45.80 ±0.02	a	1.82 ±0.05 *	1.40 ±0.08	a	0.95 ±0.04	0.89 ±0.07	a
	ONP	44.32 ±0.06	43.91 ±0.27	ab	46.28 ±0.64	44.59 ±0.15	ab	1.09 ±0.05	1.16 ±0.02	bcd	1.56 ±0.39	0.80 ±0.02	a
	Ojibway	44.08 ±0.29	43.54 ±0.12	ab	44.14 ±0.45	44.62 ±0.11	b	1.31 ±0.01	1.19 ±0.04	b	0.76 ±0.02	0.66 ±0.03	ab
	Pterophylla	43.77 ±0.12	43.42 ±0.30	ab	45.09 ±0.10	45.00 ±0.17	ab	1.18 ±0.04 *	0.99 ±0.00	bcd	0.61 ±0.04	0.62 ±0.01	b
Cultivated	Shelter	43.71 ±0.32	43.58 ±0.11	b	44.93 ±0.48	44.47 ±0.61	ab	1.44 ±0.05 *	1.06 ±0.04	b	0.95 ±0.04	0.63 ±0.02	ab
	Forestburg	44.77 ±0.21	43.47 ±0.14	ab	46.88 ±1.06	42.13 ±3.04	ab	1.15 ±0.06	1.23 ±0.12	bc	1.32 ±0.51	0.75 ±0.04	ab
	Summer	43.97 ±0.49	43.60 ±0.13	ab	45.75 ±0.19	44.92 ±0.24	ab	1.11 ±0.08	1.25 ±0.11	bc	0.57 ±0.01	0.82 ±0.01	ab
	Caddo	44.10 ±0.21	43.78 ±0.11	ab	45.60 ±0.15	45.40 ±0.25	ab	0.86 ±0.06	0.89 ±0.03	d	0.58 ±0.04	0.64 ±0.02	b
	Trailblazer	44.58 ±0.12	44.09 ±0.12	ab	46.53 ±0.81	45.54 ±0.27	a	1.02 ±0.07	1.07 ±0.04	bcd	1.26 ±0.59	0.66 ±0.09	ab
	Northern Upland	44.69 ±0.09 ◆	44.22 ±0.19	a	45.64 ±0.14	45.87 ±0.07	a	0.96 ±0.05	0.98 ±0.03	cd	0.78 ±0.12	0.69 ±0.03	ab
Wild		43.95	43.60	α	45.26 *	45.00	α	1.35	1.19	α	0.97	0.74	α
Cultivated		44.30 ◆	43.79	α	45.89 *	44.72	β	1.09	1.08	β	0.91	0.70	α

Significant differences between M-/+ treatments for all varieties determined by a two-way ANOVA at α =0.05 with no interaction between myc and variety indicated by ◆.
Significant differences between M-/+ treatments determined by a t-test at α =0.05 indicated by *.
Significant differences between varieties as determined by a Tukey's test at α =0.05 indicated by different letters.
Significant differences between cultivation level as determined by a two-way ANOVA at α =0.05 indicated by α, β.

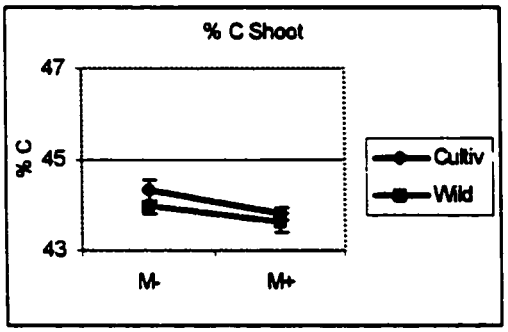


Fig 3.33. Carbon in shoot

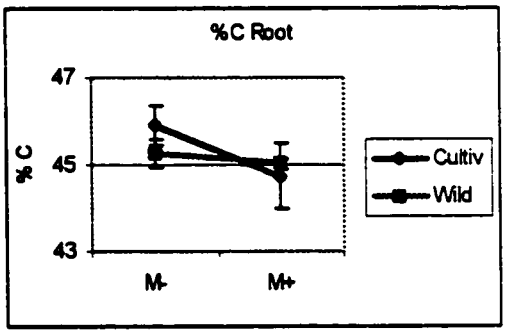


Fig 3.34. Carbon in root

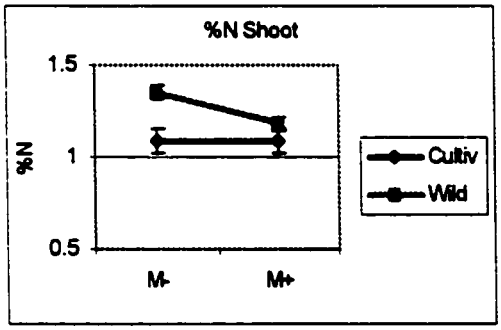


Fig 3.35. Nitrogen in shoot

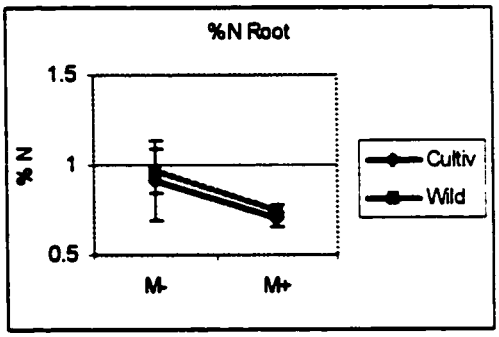


Fig 3.36. Nitrogen in root.

Figures 3.33-3.36. Total %C and %N in switchgrass of experiment 1 plants. Differences in cultivation level and mycorrhizal treatment are shown for carbon and nitrogen in roots and shoots. Standard error bars are shown. Line graphs are used to visualize the interaction between cultivation level and mycorrhizal treatment; it is not intended to suggest any continuous variable.

Table 3.11. Total percentage of Carbon and Nitrogen ± standard error for experiment 2 switchgrass plants.

	Variety	% C Shoot			% C Root			% N Shoot			%N Root		
		M-	M+		M-	M+		M-	M+		M-	M+	
Wild	Prairie Habitat	41.95 ±0.24	41.45 ±0.09	c	44.77 ±0.41	44.39 ±0.14	a	1.49 ±0.08	1.96 ±0.14	a	0.88 ±0.13	1.19 ±0.03	ab
	ONP	42.48 ±0.27	40.20 ±0.50	c	45.08 ±0.01	43.66 ±0.52	a	1.44 ±0.08	1.16 ±0.23	ab	0.93 ±0.04 *	1.18 ±0.10	ab
	Ojibway	43.57 ±0.28	43.45 ±0.62	ab	44.52 ±0.23	46.60 ±0.56	a	1.58 ±0.05	1.72 ±0.22	ab	0.92 ±0.04 *	1.49 ±0.05	a
Cultivated	Forestburg	41.72 ±0.46	43.06 ±0.46	b	45.31 ±0.07	44.27 ±0.12	a	1.24 ±0.05	1.03 ±0.06	b	0.65 ±0.03	0.75 ±0.03	c
	Caddo	43.26 ±0.05 *	44.39 ±0.20	a	44.64 ±0.35	43.85 ±0.38	a	1.12 ±0.02 *	1.76 ±0.05	ab	0.59 ±0.02 *	1.06 ±0.04	abc
	Northern Upland	42.48 ±0.18	41.55 ±0.33	bc	45.43 ±0.28	43.64 ±0.39	a	1.18 ±0.05	1.46 ±0.09	b	0.62 ±0.05	0.81 ±0.02	bc
Wild		42.67	41.70	α	44.79 *	44.88	α	1.50 *	1.61	α	0.91 *	1.29	α
Cultivated		42.49	43.00	α	45.12 *	43.92	α	1.18 *	1.42	β	0.62 *	0.87	β

Significant differences between M-/+ treatments determined by a t-test at $\alpha = 0.05$ indicated by *.
Significant differences between varieties as determined by a Tukey's test at $\alpha = 0.05$ indicated by different letters.
Significant differences between cultivation level as determined by a two-way ANOVA at $\alpha = 0.05$ indicated by α, β.

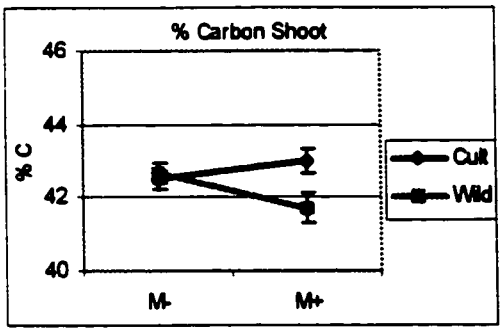


Fig 3.37. Carbon in shoots

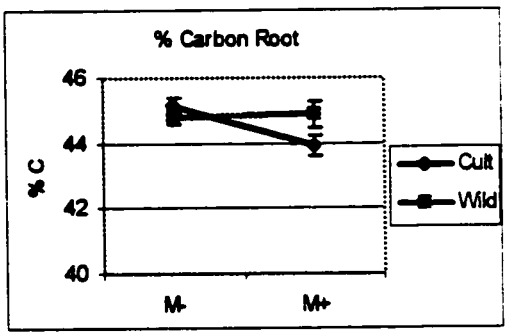


Fig 3.38. Carbon in roots.

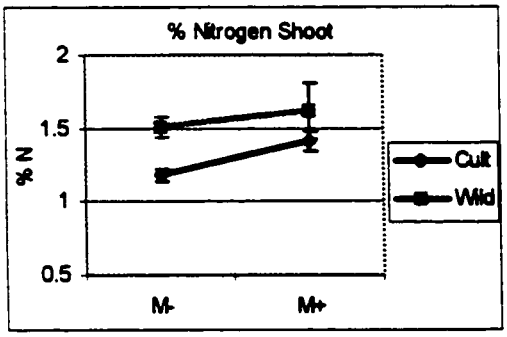


Fig 3.39. Nitrogen in shoots.

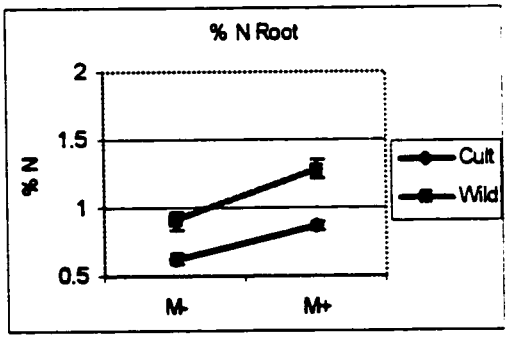


Fig 3.40. Nitrogen in roots.

Figures 3.37-3.40. Total %C and %N in switchgrass of experiment 2 plants. Differences in cultivation level and mycorrhizal treatment are shown for carbon and nitrogen in roots and shoots. Standard error bars are shown. Line graphs are used to visualize the interaction between cultivation level and mycorrhizal treatment; it is not intended to suggest any continuous variable.

Table 3.11.1. Results of two way ANOVAs at $\alpha = 0.05$ for C and N analysis of switchgrass roots and shoots. The first ANOVA was done for variety and mycorrhizal treatment, the second for cultivation level and mycorrhizal treatment.

	First ANOVA				Second ANOVA			
	R^2	V, p=	T, p=	V×T, p=	R^2	C, p=	T, p=	C×T, p=
Exp 1, n= 60								
%C Shoot *	0.632	0.001	0.000	0.340	0.239	0.050	0.001	0.648
% C Root *	0.671	0.000	0.005	0.015	0.153	0.033	0.046	0.513
% N Shoot	0.868	0.000	0.010	0.000	0.205	0.002	0.125	0.162
% N Root *	0.730	0.000	0.092	0.000	0.050	0.218	0.254	0.667
Exp 2, n= 36								
%C Shoot	0.799	0.000	0.079	0.006	0.174	0.103	0.185	0.142
% C Root*	0.684	0.029	0.002	0.003	0.240	0.247	0.014	0.175
% N Shoot	0.829	0.000	0.000	0.001	0.498	0.000	0.004	0.386
% N Root	0.921	0.000	0.000	0.000	0.752	0.000	0.000	0.091

Use of a Non-Parametric test indicated by *.
V= variety, T= mycorrhizal treatment, C= cultivation level.

Table 3.11.2. Results of two way ANOVA at $\alpha = 0.05$ for P, Ca, K and Mg analysis of experiment 2 switchgrass roots and shoots. The first ANOVA was done for variety and mycorrhizal treatment, the second for cultivation level and mycorrhizal treatment.

	First ANOVA				Second ANOVA			
	R^2	V, p=	T, p=	V×T, p=	R^2	C, p=	T, p=	C×T, p=
Exp 2, n= 36								
%P Shoot	0.841	0.000	0.569	0.000	0.150	0.033	0.774	0.431
% P Root	0.853	0.000	0.011	0.000	0.404	0.000	0.223	0.182
%Ca Shoot	0.815	0.000	0.097	0.047	0.418	0.000	0.269	0.070
% Ca Root	0.668	0.000	0.454	0.604	0.079	0.213	0.343	0.668
% K Shoot*	0.680	0.002	0.024	0.012	0.176	0.644	0.093	0.067
% K Root	0.732	0.000	0.340	0.023	0.018	0.629	0.561	0.969
% Mg Shoot	0.471	0.087	0.069	0.280	0.213	0.208	0.081	0.061
% Mg Root	0.426	0.131	0.170	0.310	0.080	0.960	0.207	0.295

Use of a Non-Parametric test indicated by *.
V= variety, T= mycorrhizal treatment, C= cultivation level

Table 3.12. Concentration of P and Ca ± standard error in experiment 2 switchgrass plants.

	Variety	P shoots (µg/g)			P roots (µg/g)			Ca shoots (µg/g)			Ca roots (µg/g)		
		M-	M+		M-	M+		M-	M+		M-	M+	
Wild	Prairie Habitat	1192 ± 24	909 ± 112	ab	961 ± 10 *	731 ± 54	a	2030 ± 64	2126 ± 239	a	921 ± 99	851 ± 25	c
	ONP	948 ± 45	909 ± 34	bc	710 ± 55	686 ± 22	ab	1393 ± 102 *	1815 ± 66	b	1011 ± 49	1144 ± 89	bc
	Ojibway	1055 ± 52	1189 ± 33	a	579 ± 13	898 ± 94	ab	1190 ± 41	1577 ± 150	bc	1247 ± 82	1287 ± 89	ab
Cultivated	Forestburg	930 ± 57 *	1243 ± 41	ab	556 ± 28 *	810 ± 40	b	1348 ± 101	1483 ± 97	bc	986 ± 162	1150 ± 61	bc
	Caddo	791 ± 46	706 27	d	481 ± 18	535 ± 40	c	1206 ± 85	1016 ± 67	c	1221 ± 168	1341 ± 26	a
	Northern Upland	951 ± 25	811 ± 42	cd	574 ± 27	586 ± 55	bc	1338 ± 65	1166 ± 138	bc	1135 ± 26	1123 ± 52	abc
Wild		1065 ± 40	1002 ± 60	α	750 ± 26	772 ± 57	α	1538 ± 69	1839 ± 152	α	1060 ± 77	1094 ± 68	α
Cultivated		891 ± 43	920 ± 36	β	537 ± 24	644 ± 45	β	1297 ± 84	1222 ± 101	β	1114 ± 118	1205 ± 46	α

Significant differences between M-/+ treatments determined by a t-test at α =0.05 indicated by *.
Significant differences between varieties as determined by a Tukey's test at α =0.05 indicated by different letters.
Significant differences between cultivation level as determined by a two-way ANOVA at α =0.05 indicated by α, β.

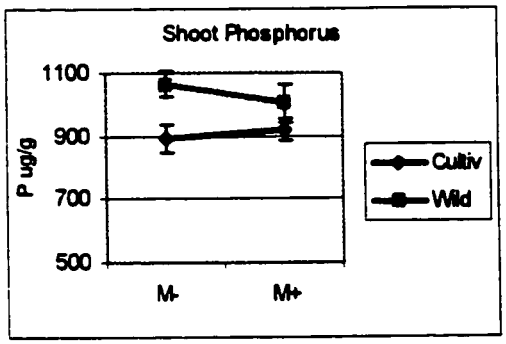


Fig 3.41. Shoot phosphorus

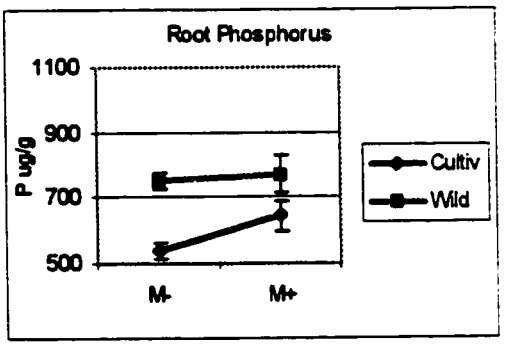


Fig 3.42. Root phosphorus

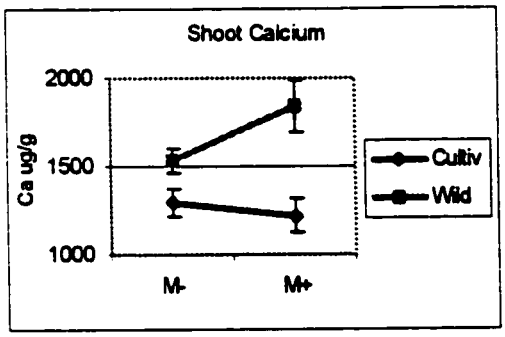


Fig 3.43. Shoot calcium

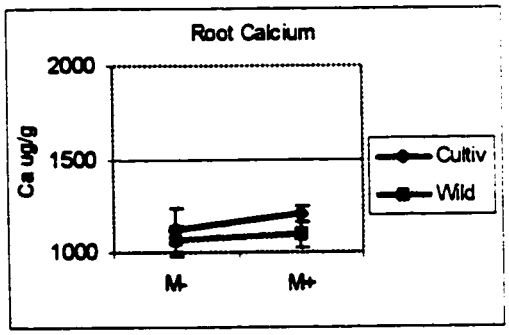


Fig 3.44. Root calcium

Figures 3.41-3.44. Concentration of phosphorus and calcium of switchgrass from experiment 2 plants. Differences in cultivation level and mycorrhizal treatment are shown for P and Ca in shoots and roots. Standard error bars are shown. Line graphs are used to visualize the interaction between cultivation level and mycorrhizal treatment; it is not intended to suggest any continuous variable.

Table 3.13. Concentration of K and Mg ± standard error in experiment 2 switchgrass plants.

	Variety	K shoots (mg/g)			K roots (mg/g)			Mg shoots (μg/g)			Mg roots (μg/g)		
		M-	M+		M-	M+		M-	M+		M-	M+	
Wild	Prairie Habitat	16.26 ± 0.73	14.63 ± 0.44	b	18.70 ± 0.95	19.55 ± 0.53	b	2018 ± 65	2258 ± 220	a	4170 ± 150	4425 ± 358	a
	ONP	18.47 ± 0.43 *	21.82 ± 0.54	a	22.95 ± 0.21	24.46 ± 1.12	a	2230 218	2800 334	a	3272 ± 152	3552 ± 235	a
	Ojibway	21.44 ± 0.53	21.63 ±0.41	a	25.27 ± 0.47	21.11 ± 2.51	a	1890 ±58	2295 ± 152	a	4555 ± 721	4171 ± 579	a
Cultivated	Forestburg	19.03 ± 1.15	18.15 ± 1.45	ab	20.31 ± 0.99	17.16 ± 0.42	b	1999 ± 132	2201 ± 182	a	3627 ± 365	4115 ± 362	a
	Caddo	21.49 ±0. 45 *	14.35 ± 1.35	ab	21.45 ± 0.29 *	23.31 ± 0.36	a	1978 ± 167	1980 ± 28	a	3377 ± 271	4633 ± 164	a
	Northern Upland	23.11 ± 0.68	19.72 ± 2.51	a	23.17 ± 0.85	23.41 ± 1.25	a	2375 ± 186	2125 ± 159	a	4236 ± 97	4089 ± 275	a
Wild		18.72 ± 0.56	19.36 ± 1.71	α	22.31 ±0. 55	21.70 ± 1.39	α	2046 ± 114	2451 ± 235	α	3999 ± 341	4050 ± 391	α
Cultivated		21.21 ± 0.76	17.41 ± 1.77	α	21.65 ± 0.71	21.29 ±0.68	α	2117 ± 161	2101 ± 123	α	3746 ± 244	4279 ± 267	α

Significant differences between M-/+ treatments determined by a t-test at α =0.05 indicated by *.
Significant differences between varieties as determined by a Tukey's test at α =0.05 indicated by different letters.
Significant differences between cultivation level as determined by a two-way ANOVA at α =0.05 indicated by α, β.

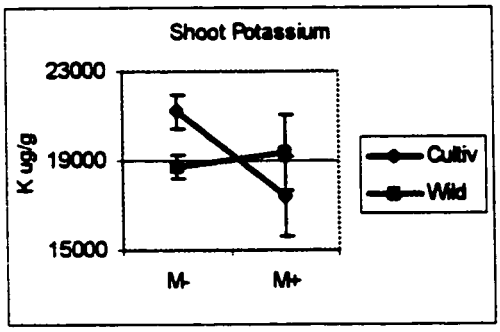


Fig 3.45. Shoot potassium.

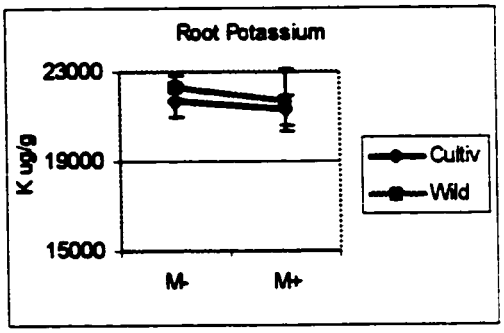


Fig 3.46. Root potassium

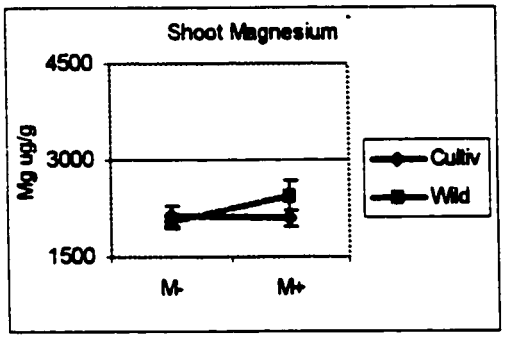


Fig 3.47. Shoot magnesium.

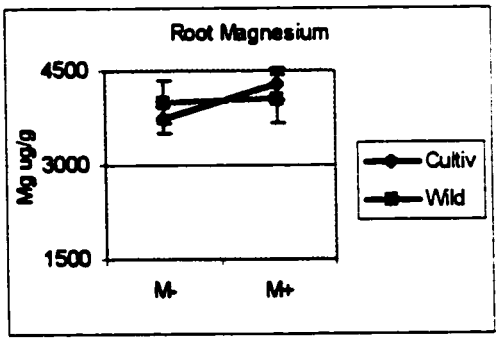


Fig 3.48. Root magnesium.

Figures 3.45-3.48. Concentration of potassium and magnesium of switchgrass from experiment 2 plants. Differences in cultivation level and mycorrhizal treatment are shown for K and Mg in shoots and roots. Standard error bars are shown. Line graphs are used to visualize the interaction between cultivation level and mycorrhizal treatment; it is not intended to suggest any continuous variable.

10.0 – Statistical Analysis

10.1- Cluster Analysis

First Experiment

Two main clusters separate the varieties in the first experiment (Fig 3.49). Clusters seem to separate the varieties that responded positively to the mycorrhizal treatment, from varieties that responded negatively. Cluster 1, the positive responders, includes NU, Summer, Forestburg and ONP. Cluster 2 includes PH, Shelter, Ojibway, Trailblazer as well as Pterophylla and Caddo, which are closely joined to each other and separate from the other varieties in this group. More varieties had negative than positive responses in this experiment. Both Forestburg and ONP, which were closely joined, had positive responses to mycorrhizae almost consistently. Northern Upland and Summer, the other varieties included in this cluster, had positive responses for some of the variables and not for others. Positive effects were found in height, fresh mass, a decrease in root diameter, and an increase in % N shoot that were greater than those of the other varieties. Of the negatively responding group, Caddo and Pterophylla had negative responses in mass, increases in root diameter and a drop in acid phosphatase level, which set them apart from the others. The remaining varieties were very closely joined to each other, and in other types of joining their positions switched. Trailblazer, Shelter, Ojibway and Prairie Habitat had large negative responses in fresh mass, and varied responses to all the other factors. There were no distinct groupings between the wild and cultivated varieties.

Second Experiment

The two main clusters were separated seemingly on root characteristics for the cluster analysis on the second experiment (Fig 3.50). Although two out of the three from the wild and cultivated groups clustered closely together, Forestburg grouped with the wild types and Ojibway with the cultivars. All three of the first cluster, Forestburg, ONP and PH have significantly higher root:shoot ratios than the others. Differences between clusters in effect size were noticed for fresh weight where group 1 had high positive values while the others were negative. No other obvious segregations were noticed.

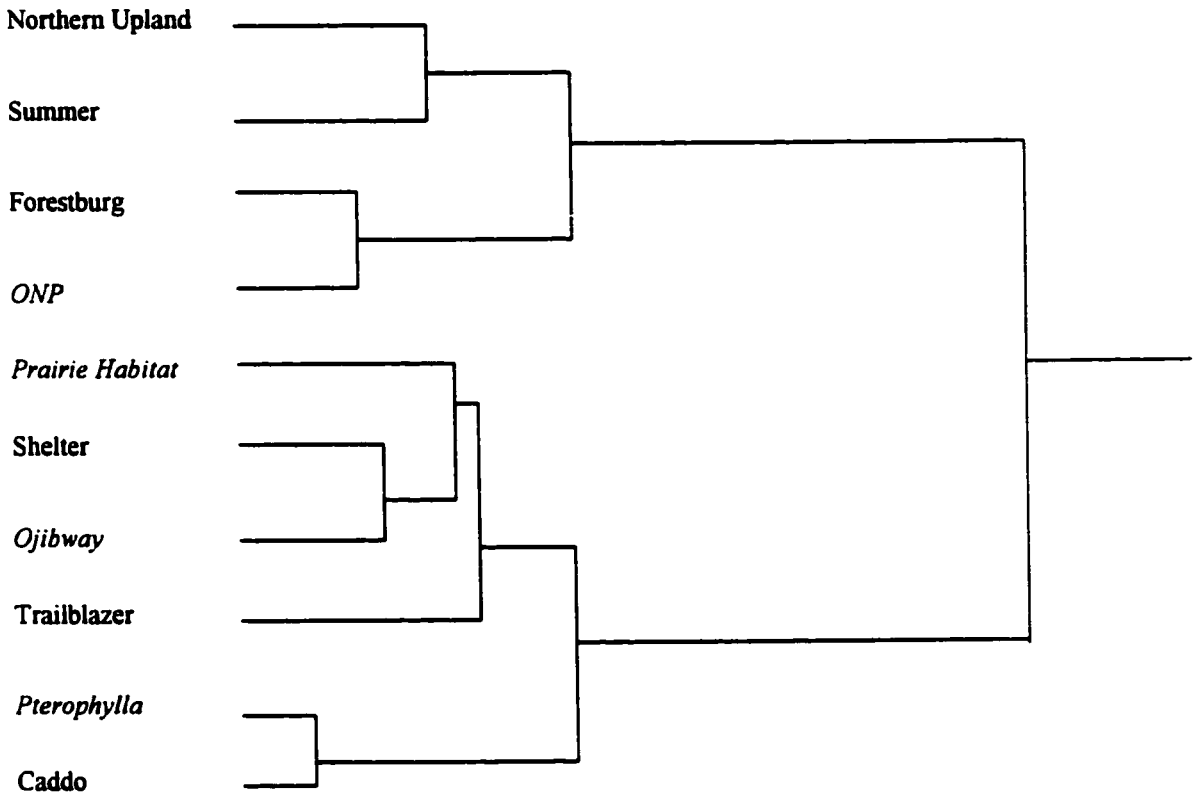


Figure 3.49. Cluster analysis of the mycorrhizal treatment effect size on wild and cultivated varieties of switchgrass for the first experiment plants using Ward joining. Wild varieties are indicated in italics.

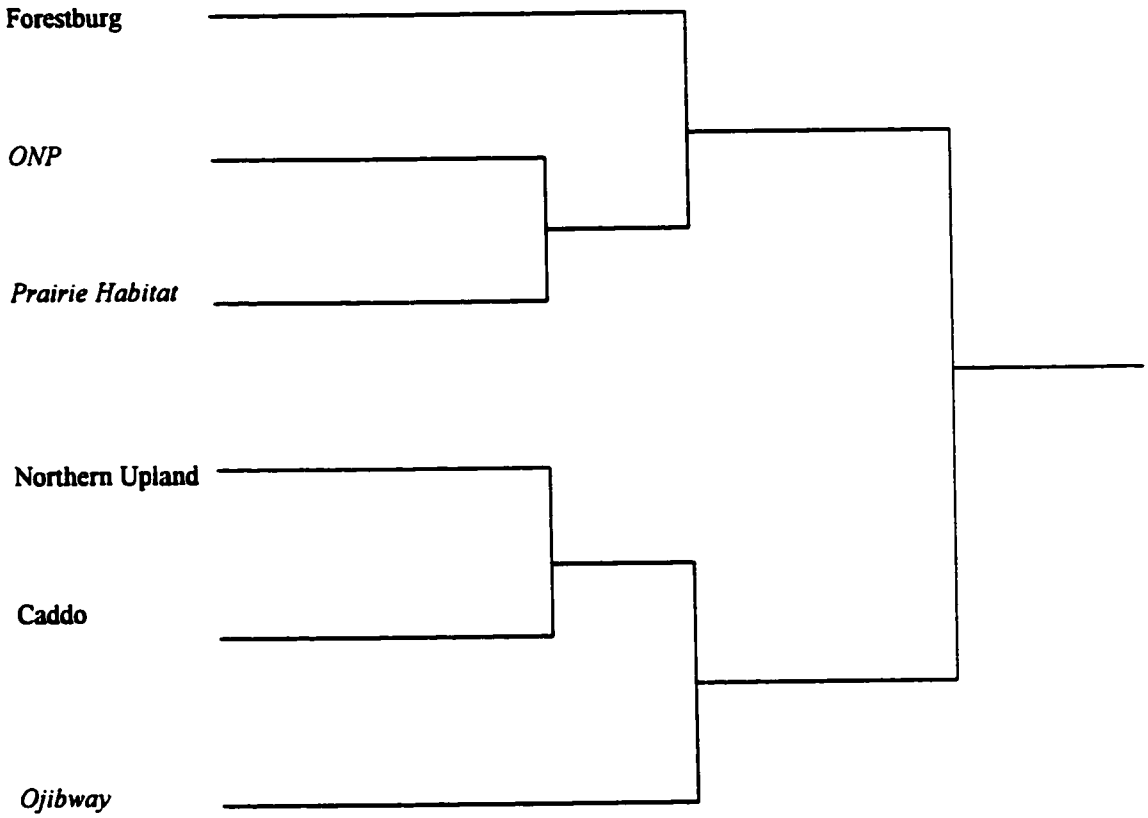


Figure 3.50. Cluster analysis of the mycorrhizal treatment effect size on wild and cultivated varieties of switchgrass for the second experiment plants using Ward joining. Wild varieties are indicated in italics.

10.2- Canonical Discriminant Analysis

Experiment 1

To assess the validity of the patterns found in the cluster analysis, a canonical discriminant analysis (CDA) was performed. Three clusters were identified for this analysis. Cluster 1 included NU, Summer, Forestburg and ONP; cluster 2 included PH, Shelter, Ojibway and Trailblazer; cluster 3 included Pterophylla and Caddo (Fig 3.51). The clusters were separated in this manner because two clusters (cluster 1 and clusters 2&3) had a lower predictive rate than 3 clusters. Stepwise discriminant analysis was done to determine which factors were the most important to cluster grouping. Results from the stepwise CDA showed that 4 variables were the best predictors of cluster grouping. The most important predictors of clustering by effect size were fresh mass, acid phosphatase level, average root diameter, and % of small roots (Table 3.14). The clusters were significantly different from each other using Wilk's lambda, while predictive ability using jackknifed classification, as well as eigenvalues are very good in this analysis (Table 3.15).

Experiment 2

CDA was performed to determine validity of the clusters in experiment 2. Cluster 1 was comprised of Forestburg, ONP and PH; cluster 2 of NU, Caddo and Ojibway. From the forward stepwise CDA (F-to-join values), it was determined that two variables were important in determining clusters. The most important factors were determined to be fresh mass and root length (Table 3.16). Cluster groups were significantly different using Wilk's lambda, predictive values (jackknifed classification %) were good but the eigenvalues were lower in this experiment than in the first because of the smaller sample size (Table 3.17). Only one canonical axis was generated since only two clusters were compared. Canonical scores grouped by cluster are displayed in figure 3.52.

Table 3.14. Mean effect size values for each cluster in experiment 1.

	Cluster 1	Cluster 2	Cluster 3
Fresh Mass	4.426	-10.598	-11.956
Acid Phosphatase	-2.030	-2.159	-4.386
Average Diameter	-3.587	-1.984	2.580
% Small Roots	-0.213	1.823	1.350

Table 3.15. Statistics from canonical discriminant analysis of experiment 1 clusters.

Statistic		Value
Wilks Lambda		p = 0.00000
F-to-Remove	Fresh Mass	144.87
	Acid Phosphatase	90.75
	Average Diameter	10.68
	% Small Roots	4.94
Jackknifed Classification		100%
Eigenvalue		342.39
Canonical Correlation		0.999

Table 3.16. Mean effect size value for each cluster in experiment 2.

	Cluster 1	Cluster 2
Fresh Mass	8.151	-5.869
Root Length	2.775	0.238

Table 3.17. Statistics from canonical discriminant analysis of experiment 2 clusters.

Statistic		Value
Wilks Lambda		p = 0.0021
F-to-Remove	Fresh mass	113.65
	Root Length	8.56
Jackknifed Classification		100%
Eigenvalue		59.31
Canonical Correlation		0.992

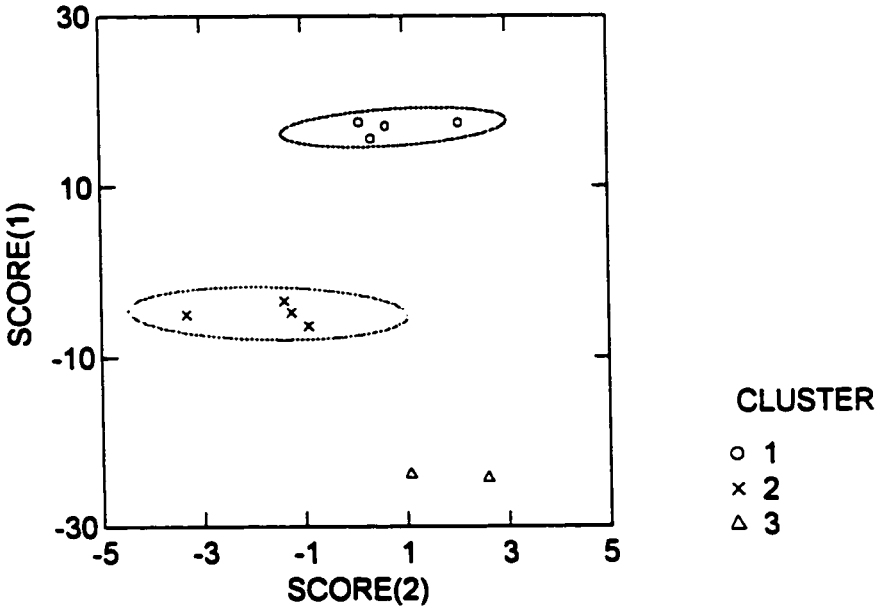


Figure 3.51. Canonical scores of clusters as determined by canonical discriminant analysis of experiment 1 switchgrass plants. Cluster groups are surrounded by p= 0.60 confidence ellipses where statistically possible.

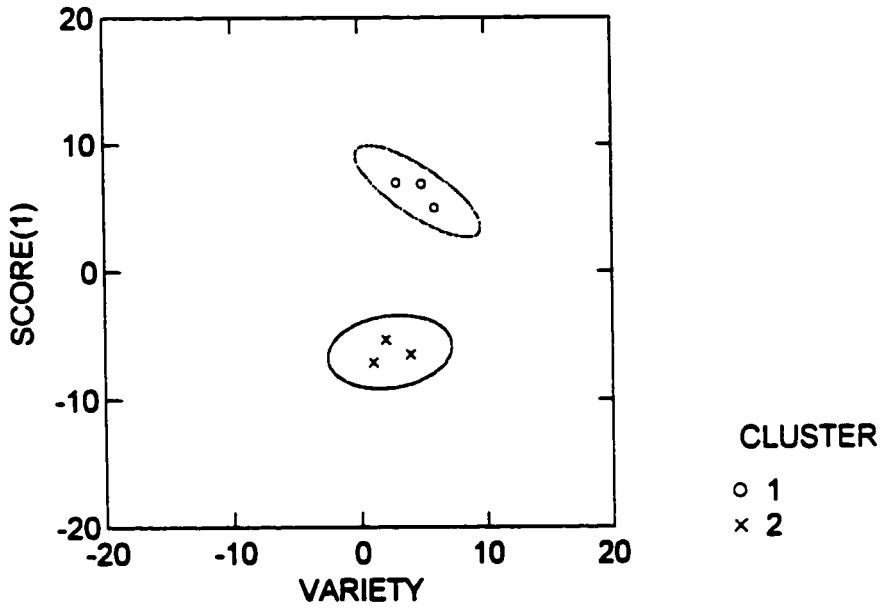


Figure 3.52. Canonical scores of cluster groups as determined by canonical discriminant analysis of experiment 2 switchgrass plants. Cluster groups are surrounded by p= 0.60 confidence ellipses.

Discussion

1.0- Switchgrass Varieties

1.1- Physiological Differences Among Varieties

The cultivars of switchgrass showed some signs of genetic selection for height and mass. All the cultivars were larger than their wild counterparts. Cultivars NU and Caddo were very tall, sparsely branched and had a high biomass. There was more variation among the wild varieties in their physiology. The wild variety PH grew to a low height but was highly branched and produced many tillers. In contrast, Ojibway was mid height and slightly branched, while ONP, a wild variety, was tall and similar to cultivars. Other researchers have also observed variation in appearance, tillering, branching patterns, forage quality, and other physiological characteristics among switchgrass varieties or populations (Nielsen, 1944; Eberhart and Newell, 1959; Hopkins et al., 1995; Madakadze et al, 1998b).

The plants in this greenhouse study did not grow to the usual height of switchgrass, and they also started to flower early. This stunting is most likely caused by growth stress from being in a small soil volume and nutrient stress caused by the low fertilization level.

The height of the tallest leaf was found to be an adequate measure of growth for grass plants in this experiment and in others (Miller et al., 1987). Plants experienced linear growth over time, for the most part. Fluctuations in the growth rate tend to coincide with fertilizer inputs; increases were noted after fertilization. After an 8-week growth period,

many varieties seemed to experience a plateau. The height of cultivars Forestburg and Summer, and wild varieties ONP and PH noticeably level off. It is possible that at this point, their root systems became limited by the cones in which they were grown. Interestingly, the varieties that had the longest root lengths were not the ones that experienced the growth plateau. The varieties that levelled off in growth had in common the fact that they generally benefited from mycorrhizal colonization. Growth limitation could be due to the soil volume being fully exploited by the hyphal network. The growth rate of cultivars was higher than that of wild varieties. Faster growth of cultivars may reduce their potential to experience biomass and height benefits from mycorrhizal colonization, since most plants experience a growth depression when first colonized (Smith and Read, 1997).

Chlorophyll levels of the first experiment plants did not differ much although significant differences were found. A large sample size can reduce the standard error and give statistically significant results when the actual differences are minimal in a biological perspective. The slightly higher values seen in wild plants can be explained since higher chlorophyll concentrations were found in plants with lesser biomass. Resources such as chlorophyll can become diluted in plants that have a large volume (Madakadze et al., 1999). Forestburg, which had the highest chlorophyll concentration, does not fit this assumption as it had a fairly large biomass. The chlorophyll meter readings were lower than those found in field stands of switchgrass fertilized with high N rates (Madakadze et al., 1999b). The lack of response in chlorophyll levels to mycorrhizal colonization was also found in turfgrasses (Charest et al.,1997) and maize (Subramanian and Charest,

1995). Other researchers have found increases in chlorophyll levels when plants are mycorrhizal (Allen et al., 1981).

1.2- Chromosome Numbers

The ploidy of switchgrass varieties found in this study in most cases did not concur with the amount of chromosomes expected. Other studies have provided conflicting reports on the ploidy of several of the cultivars used in the present study. The chromosome numbers of varieties that are either hexaploid or octoploid are very difficult to distinguish in dividing root tips because of their small size. In this study, the wild variety ONP actually had been counted with 2 ploidy levels, 54 and 72. It has been suggested that octoploid varieties may experience 'stickiness' during mitosis that makes these chromosomes even more difficult to count (Dr. Ken Vogel, pers. comm.). Contrary to expectations (McMillan and Weiler, 1959), the wild varieties in this study were all found to be hexaploid and not tetraploid. There may be several reasons for this discrepancy, the ploidy of the plants might have changed since 1959 or Canadian varieties, which were not included by McMillan and Weiler, are different ecotypes or the wild varieties might have interbred with cultivated types. Ploidy level does not seem to correspond with any physiological character such as height, biomass or tillering in this study.

2.0- Mycorrhizal Response

2.1- Colonization

The levels of colonization of switchgrass roots with *Glomus intraradices* found in this study did not vary significantly among varieties or between the two greenhouse experiments. The average colonization level of 36% corresponds to the levels found for switchgrass in other studies, under both greenhouse and field conditions (Hetrick and Bloom, 1983; Brejda et al., 1993; Johnson, 1998). It is possible that ~ 40% is the threshold colonization level for switchgrass.

Various studies on the responsiveness of switchgrass to AM fungi have shown that a certain level of fungus-plant compatibility may occur. Different AM fungal species were found to vary widely in their relative spore numbers when associated with switchgrass (Hetrick and Bloom, 1983). This suggests that some AM fungal species may survive better than others when associated with switchgrass in the wild. Species diversity of AM fungal spores was found to be low in the vicinity of switchgrass plants in comparison with the diversity under other tallgrass prairie species (Eom et al., 2000). This suggests that switchgrass may be a more compatible host for some AM fungi than others. Plant response to AM fungi varies as well, suggesting that the host plant also has some preference in its symbiotic partner. Brejda et al. (1993) found that an unspecific inoculum that contained many species of AM fungi produced more benefits to switchgrass than did a pure isolate of *Glomus deserticola*. When switchgrass was grown with several separate

species of mycorrhizal fungi, *Glomus clarum* and *Glomus diaphanum* were the most effective species at increasing plant biomass while *Glomus intraradices* was less compatible (Clark et al., 1999; Eom et al., 2000). However, positive growth responses were observed in switchgrass plants colonized with *G. intraradices* when compared to control plants (Clark et al., 1999). It is possible that the lack of many significant mycorrhizal responses in this study was due to a relatively low symbiotic compatibility of *Panicum virgatum* with *Glomus intraradices*.

It has been suggested that plants with higher ploidy levels may have higher mycorrhizal colonization levels. In the present study, no differences in colonization percentage were found among the varieties, regardless of their ploidy. Differences between cultivars of various ploidies were found by Jun and Allen (1991), but no clear relationship between ploidy and colonization was found. Kapulnik and Kushnir (1991) found that polyploid wheat had higher % colonization than diploid wheat; however this was not related to mycorrhizal efficiency. Berta et al. (2000) found that tomato cultivars with higher ploidies had higher colonization levels.

2.2- Physiological Responses to Mycorrhizae

Greenhouse growth conditions were important in determining plant response to mycorrhizal colonization. In the first experiment, where soil volume was limiting, a negative response was seen for almost all the varieties. Therefore, in the first experiment of this study, the carbon cost is likely to have exceeded the benefits of AM fungal symbiosis. The soil volume was increased in the second experiment, and the responses to

colonization became either neutral or positive. Baath and Hayman (1984) also noted that mycorrhizal symbiosis in onion (*Allium cepa* L.) with both *Glomus mosseae* and *G. caledonia* went from beneficial to antagonistic when soil volume was reduced. In small soil volumes it is likely that the root system of the plant is sufficient for mineral absorption.

Biomass is often used as the benchmark of mycorrhizal response. In this study, mass differences due to mycorrhizae were not as dramatic as others reported in the literature (reviewed in Smith and Read, 1997). Biomass decreases observed in the first experiment were not unexpected, when the stressful growth conditions are taken into account. The responses of the host plants seen in the second experiment are more reliable than those of the first experiment; therefore, the second experiment plants are considered more closely. The cultivated group had a larger biomass response to mycorrhizal colonization, mainly because of growth increases in Forestburg. Dry mass decreases in the wild varieties PH and Ojibway, the two smallest varieties, also contributed to this difference. A similar study by Clark et al. (1999) found the total dry mass of switchgrass decreased when colonized by *Glomus intraradices* at pH 4; the present study was performed at pH 4.5. However, most studies with switchgrass reported biomass increases when mycorrhizal (Hetrick et al., 1988; Bredja et al., 1998; Johnson, 1998; Entry et al., 1999).

Root to shoot ratio is a good measure of plant health, when compared within a species, and life history, when compared between species. A large R/S indicates a higher investment in underground structures for resource gathering. The R/S usually increases if

a plant is mineral or water stressed (Hopkins, 1995). The average R/S of near 1.0 in the first greenhouse experiment indicates the stressed condition of these plants. The R/S decreased to 0.5 in the second experiment. In both experiments of this study, the soil pH was 4.5. The R/S in the second experiment is similar to what was found in the study of Clark et al. (1999), where switchgrass colonized by *G. intraradices* had R/S ratios of 0.58 in soil at pH 4 and 0.35 at pH 5.

Since the hyphal network serves the same function as small roots, fewer roots need to be produced and the R/S should decrease in mycorrhizal plants. The R/S was found to be higher in mycorrhizal plants of the first experiment of this study. This is likely because of increased stress level and C transferred to AM fungi, making the shoots grow more slowly (Tinker et al., 1994). This R/S increase was more pronounced in wild plants, which already invested more into root growth. The wild varieties continued to allocate more resources for root growth, but both wild and cultivated groups maintained a steady R/S under M+ and M- treatments in the second experiment. Bredja et al. (1993) found that switchgrass responded to mycorrhizal colonization by decreasing R/S. This trend has been observed in many species such as onion (Baath and Hayman, 1984), wheat (Vierheilig and Ocampo, 1991), *Salix viminalis* L. (Tinker et al., 1994), and lettuce (Koide et al., 2000). Cultivated varieties need to invest fewer resources towards root growth since they are less likely to experience nutrient or drought stress than wild plants. Lower R/S in cultivars may be a sign that agricultural selection has changed the physiology of switchgrass. Shoot biomass is a characteristic selected for in breeding programs, so it is not unexpected that shoots are more emphasized in the cultivated

group. It is interesting to note that Forestburg, a cultivar that has not undergone selection, maintains a high R/S while Ojibway, a wild variety, has a fairly low R/S.

In grasses, growth can occur either vertically through the stem and leaves, or laterally via tillers. While height was consistently greater in the cultivated plants, tiller numbers were not different between the cultivation levels when non-mycorrhizal. An interesting phenomenon was found in this study: a significant decrease in tiller numbers occurred in mycorrhizal plants of both experiments and cultivation levels. The only variety unaffected by this decline was the cultivar Forestburg, which seems to compensate by having a lower height when mycorrhizal. However, no correlation between height and tiller number was found in this study. A lack of vegetative reproduction could indicate that the plants were stressed by the growth conditions. Our results are contrary to those of Bredja et al. (1993) who observed increased tiller numbers in mycorrhizal switchgrass. Mycorrhizae increased tiller numbers but decreased height of the prairie grass *Agropyron smithii* L., while an overall mass increase was noted (Miller et al., 1987). Overall, mycorrhizae did not affect the height of plants in the second experiment. Height increases due to mycorrhizal colonization were observed in switchgrass by Johnson (1998).

In the first experiment, under stress conditions, the wild varieties experienced smaller declines in mass, height and tillers than the cultivars when mycorrhizal. ONP had the effect of buffering the detrimental mycorrhizal effects in the collective results of the wild varieties, since Ojibway and Pterophylla both had significant declines for most of the physiological parameters examined. Cultivars Caddo, Summer and Trailblazer declined

significantly for almost every physiological parameter when colonized by mycorrhizae. In the second experiment, under less limiting growth conditions, the wild variety ONP and the non-selected cultivar Forestburg benefited from mycorrhizal colonization while the other varieties had neutral or negative responses. The agriculturally selected cultivars, Caddo and NU, both had declining mass and tiller numbers, suggesting negative physiological responses of cultivars to mycorrhizal colonization.

2.3- Root Architecture Responses to Mycorrhizae

Since mycorrhizal hyphae are in some ways a replacement for root hairs, root architecture is an important factor in determining mycorrhizal response (Baylis, 1970; Manjunath and Habte, 1991). As well, mycorrhizal colonization can induce changes in roots that may determine the symbiotic efficiency. Since mycorrhizal symbiosis seems to be the ancestral condition in plants, root morphology that lowers mycorrhizal dependency suggests an environmental adaptation. Special physiological adaptations such as bulbous root hairs were related to the non-mycorrhizal state of some Carex species (Miller et al., 1999). Grasses have adventitious root systems, which are highly branched and have small average diameters. Because the existing root systems should be able to adequately exploit the soil volume without the aid of the mycorrhizal hyphal network, mycorrhizae have been theorized to be neutral or commensal symbionts for grasses (Francis and Read, 1995). Nonetheless, many grass species, especially C4 grasses including *Andropogon gerardii* Vitm., *Sorghastrum nutans* L., and *Bouteloua cutipendula* Michx., are highly mycorrhizal dependent (Hetrick et al., 1988,1990 &1992).

An increase in total root length was observed in the mycorrhizal plants in both experiments. Root length increases due to AM mycorrhizal fungi have also been observed in other studies with switchgrass (Hetrick et al., 1991; Clark et al., 1999), and with *Plantago lanceolata* (Hodge et al., 2000). Schweiger et al. (1995) found no differences in either root length or diameter between mycorrhizal and non-mycorrhizal treatments in some pasture species. It is possible that increased root length occurs because resources no longer need to be allocated to root hairs and branching.

In this study, large differences in root diameter were detected between varieties in the first experiment, while the root diameters were smaller and fairly similar among varieties in the second experiment. Cultivars were found to have larger root diameters than wild varieties in both experiments. Since plants with coarser roots would find functional complementarity with the fine mycorrhizal hyphae, it is predicted that they should benefit most from the symbiosis. Fine roots and mycorrhizae were suggested to be alternative methods of acquiring minerals (Gahoonia et al., 1999), with the former being at a selective advantage under agricultural conditions. Switchgrass cultivars do not seem to be lowering their mycorrhizal dependency by producing finer diameter roots. Root diameter did not affect the % colonization of roots in this study, in contrast to the study of Reinhardt and Miller (1990) where mycorrhizal colonization decreased as average root diameter decreased. The average root diameter should theoretically increase when plants are colonized by AM fungi, since small diameter absorptive structures would be redundant. In this study, root diameter only increased in the mycorrhizal plants of the cultivars in the second greenhouse experiment. Root diameter increases in mycorrhizal

plants were found in several warm season grasses including switchgrass, tall grama-grass, indiangrass, big bluestem, and little bluestem Hetrick et al. (1991). Kaldorf and Ludwig-Muller (2000) found that smaller diameter roots increased in maize plants colonized by *Glomus intraradices*, possibly because of the effect of a growth hormone (indole-3-butyric acid) and its derivatives.

Root tip number, as a measurement of branching, was not as good a determinant of mycorrhizal effect as expected. Increased root branching in the mycorrhizal plants was noted in the first experiment and no differences in root tip numbers were observed in the second. It is likely that sub-optimal growth conditions in the first experiment caused stress, which induced increased branching. However, Hetrick et al. (1991) found that mycorrhizae suppressed root branching in C4 grasses. The determination of % large roots did not reveal any interesting results. There was a very small amount of roots with large diameters, and no clear patterns emerged from this variable. The % of small roots is more interesting. Schweiger et al. (1995) found that the length of root hairs was more important in determining mycorrhizal effect than root length, diameter and R/S. Graminaceous plants have adventitious root systems; therefore it is expected that most of the roots would have small to medium root diameters. As this category of roots would be directly replaced by mycorrhizal hyphae, their % of the total root length should decrease when colonized. Also, when plants are stressed they tend to produce more root hairs and small diameter roots. The increase in % small roots seen in some of the first experiment varieties is most probably due to a stress response. The response in small root production turns out to be a determining factor in the clustering of the first experiment varieties. In

the second experiment, when mycorrhizae were more effective, an overall decrease in the % of small roots was measured for all the varieties.

Root architecture has been used as a predictor of mycorrhizal dependency in many studies (Manjunath and Habte, 1991; Khalil et al., 1994). Criteria for mycorrhizal dependency list low R/S, little branching, large root diameter and root plasticity as factors that should indicate high responsiveness (Smith, 2000). Root plasticity refers to the variation in root morphology of a species or genotype and its ability to adapt its physiological parameters, such as root length, branching or diameter, to specific environmental conditions. In this study, wild varieties seemed to show more plasticity in their root morphological responses to mycorrhizal colonization. According to the scheme of Smith (2000), the cultivar Caddo should be the most mycorrhizal responsive variety, but this was not the case. The cultivar Forestburg had the highest MD, it also had high R/S, a large root length, and large diameter.

2.4- Mineral Responses to Mycorrhizae

Average mineral concentrations (μg/g) found in this study were P- 970 shoot, 676 root, Ca –1474 shoot, 1118 root, Mg –2179 shoot, 4018 root, K –19,175 shoot, 21,738 root. Mineral concentrations in μg/g for mycorrhizal switchgrass shoots at pH 4-5 were reported to be: P 100-1500, Ca 1000-8000, Mg 1000-1500, K 3000-25 000 (Clark et al., 1999). Concentrations are within the normal range for all minerals except for high

magnesium contents. High magnesium contents (890-3370 μg/g) were also noted in switchgrass under fertilized field conditions (Jung et al., 1988).

Varietal differences were found for all minerals except for magnesium. Highest mineral levels were found in wild varieties, possibly because of their smaller biomass. Caddo continuously had the lowest mineral content, as well as showing increases when mycorrhizal. This could indicate that it has low mineral requirements.

Plant mineral content is expected to increase with the addition of mycorrhizal fungi to the soil microflora (Gianinazzi-Pearson and Gianinazzi, 1983; Smith and Read, 1997). The effect is most pronounced when minerals are sparse in the soil environment. The plants in both experiments of this study were put on a low nutrient fertilizer regime and so were reasonably mineral stressed in order to emphasize the mycorrhizal effect. The P levels measured in this study were lower than those reported for switchgrass under field conditions (Jung et al., 1988). The only variety to experience an increase in P content with mycorrhizae was Forestburg, while PH experienced a decrease in P. Levels of K increased in ONP and Caddo, and Ca increased in ONP. Overall, mycorrhizae did not seem to increase uptake of minerals to any great extent. A study using the switchgrass cultivar 'Cave-in-Rock' found that P, K and Cu increased when *Glomus intraradices* was introduced (Clark et al., 1999). Johnson (1998) found P levels increased in switchgrass inoculated with three *Glomus* species (including *G. intratradices*); in addition P content was correlated with mass and height, effects not detected in this study. Increased mineral

concentrations were also observed in switchgrass colonized by *G. intraradices* in the study of Entry et al. (1999).

Concentrations of N in switchgrass are often reported to be around 10 g/kg or 1% by weight (Staley et al., 1991; Madakadze et al., 1999). Lower N concentrations of 5.1-9.2 g/kg or higher concentrations of 5.4-20.2 g/kg may also be found, depending upon variety and N availability in the soil (Madakadze et al., 1999c). In the field, N uptake tended to plateau early when fertilizer application was very high (Staley et al., 1991). It is common to find that the % N and other minerals decrease throughout the growing season as they are diluted through the plant tissue (Talbert et al., 1983; Balasko et al., 1984; Madakadze et al., 1999). A decrease in crude protein corresponding to that of N, was reported by Anderson and Matches (1983).

Nitrogen concentrations in this study (0.58-1.96%) were comparable to field values despite the low fertilization regime. The highest N concentration was found in the wild variety PH, which had the smallest mass. This result may correspond with the dilution effect mentioned by Madakadze et al. (1999). The mycorrhizal treatment tended to have opposite effects on % N in the two experiments. A decrease was found in the first experiment while an increase was observed in the second. The model proposed by Bago et al. (2001) on N translocation from mycorrhizal fungi to roots leaves allowance for N to move back into the fungus, and tends to link N movement to C flux. If the fungus was stressed for either C or N in the first experiment, translocation of both would have

decreased, which was observed. Increases in N with mycorrhizae in the second experiment were significant for the wild varieties ONP, Ojibway and the cultivar Caddo.

Wild varieties had higher acid phosphatase levels, except for the non-mycorrhizal plants of the first experiment; high levels in cultivars Shelter and Trailblazer skew the results of this treatment. This result is not surprising as it is more likely that wild plants would need to convert inorganic P from rock phosphorus than plants that receive PO_4 in fertilizer. No clear pattern emerges between the MD of plants and acid phosphatase levels in this study. The correlation between acid phosphatase level and P concentration was poor (non-significant), even though acid phosphatase should increase P uptake and therefore internal concentration. The contribution of mycorrhizal fungi to acid phosphatase levels has been found to depend on the AM fungal species (Boddington and Dodd, 1998). Acid phosphatase levels in this study were found either to decrease in the mycorrhizal treatment or to remain constant. Other studies attempting to determine mycorrhizal contribution to acid phosphatase have come up with contradictory or non-significant results (Gianinazzi-Pearson and Gianinazzi, 1976; Rubio et al., 1990). Acid phosphatase has also been reported to increase up to 60% with the addition of mycorrhizae (Tarafdar and Marschner, 1994; Khalil et al., 1999). Decreased production of acid phosphatase has been linked to higher phosphorus use efficiency (McLauchlan, 1980) and increases indicate a deficiency of P in the plant (Marschener, 1998). In this study, wild varieties of switchgrass had higher levels of acid phosphatase than the cultivars. This is in contrast to the findings of Khalil et al. (1994) where wild varieties of corn and soja had lower acid phosphatase levels and higher MD.

2.5- Cost/Benefit Analysis

Carbon content in plants can be used as a measure of their energetic status. As mycorrhizae are acting as a carbon sink for the plant, the amount of shoot carbon may decrease. It is known that mycorrhizae induce the plant to divert C resources from the shoots to the roots after colonization (Douds et al., 1988; Graham et al., 1997). Instead of the expected increase as C is diverted to the roots, root C levels decreased with in the mycorrhizal treatment in the present study. A decrease in % C was also noted by Entry et al., (1999) in switchgrass colonized with *G. intraradices*, but this did not deter biomass increases in the plant. It is possible that we are not detecting the entire carbon pool available to the roots and fungi, as the hyphal network could not be analyzed. The cost of the symbiosis may be exceeding the benefits to the plant in this particular case, even though the % colonization observed was not excessive. Carbon limitation, due to a large carbon sink or low production of photosynthate for example, is one of the conditions that can cause the symbiosis to be antagonistic to the plant (Johnson et al., 1997; Smith, 2000).

ONP, a wild variety, and Caddo, a cultivar, seem to have overall improved mineral uptake when mycorrhizal. Caddo did not show any physiological effects of this improvement in mineral nutrition, but ONP did have growth increases when mycorrhizal. Forestburg showed increased mineral concentration with mycorrhizae only for P, but did show positive mycorrhizal responses by increasing physiological parameters such as mass and root length. These data suggest that, in this study, improved mineral nutrition was not the sole causal factor for plant physiological responses to mycorrhizae. Mineral

uptake was also found to be distinct from biomass increase in Hetrick et al. (1996). The high cost of symbiosis under greenhouse growth conditions may be contributing to the lack of physiological benefits observed. In this study, a relationship between C cost and mineral gain was not observed.

The level of benefit that plants gain from mycorrhizal colonization depends upon the environmental conditions. Plants grown in soil with a high mineral content tend to show less benefit or even depressed growth under mycorrhizal conditions (Menge et al., 1978; Francis and Read, 1995). The phenomenon of high soil P levels having a negative effect on mycorrhizal colonization is widely known. A list of hypotheses for plant control of colonization mediated by P availability has been reviewed in Schwab et al. (1991). A recent model (Bago et al., 2000) proposes that P transport into host cells is linked directly to carbohydrate transfer to fungal hyphae through a common translocator. Such a system would limit the amount of carbohydrate spent on mycorrhizal symbiosis in excess mineral conditions, keeping their cost/benefit ratio manageable.

Responses to mycorrhizal colonization varied depending upon variety of switchgrass and experimental conditions. Responses ranged from negative to positive, spanning the symbiotic continuum. The ability to show a range of responses within a symbiotic association is fully described by Bronstein (1994). Selective breeding under fertilized agricultural conditions would tend to propagate genotypes that can respond quickly and strongly to increased mineral availability, thus their response to mycorrhizae would be very sensitive to soil mineral availability. Wild plants tend to have slower growth and

responsiveness to mineral availability. Plants in natural environments are more likely to maintain mycorrhizae, even under conditions when symbiosis is detrimental, in order to gain the benefits of mineral nutrition and stress reduction under certain environmental conditions. Continuing to maintain mycorrhizal symbiosis even when the carbon cost is higher than the mineral benefits has been theoretically modeled as a plausible evolutionary strategy if the potential exists for a beneficial relationship when conditions change (Herre et al., 2000; Tuomi et al., 2001). Under agricultural conditions with high fertilizer input, quick responsiveness of plants is favoured, and plant mycorrhizal colonization would be reduced, even eliminated. Symbiotic associations are less likely to be maintained in agriculture; therefore, wild plants are theoretically more likely to be mycorrhizal dependent.

2.6- Mycorrhizal Responsiveness of Switchgrass Varieties

In this study, the varieties with the overall most positive responses to mycorrhizal colonization are Forestburg, a non-agriculturally selected cultivar, and ONP, a wild variety. They cluster closely together in both experiments. PH, a wild variety that clusters in this group, is not responsive to mycorrhizae, except for a decrease in root P. The cultivars NU and Caddo group together in the second experiment cluster analysis and are generally not responsive to mycorrhizae, except for positive responses by NU in dry mass and root diameter and by Caddo in C, N, and K levels. The wild variety Ojibway that clusters with this group seems to have a negative response to mycorrhizal colonization in both experiments, especially in root parameters. Cluster analysis on effect size does seem

to be adequate for separating varieties based on an overall MD. Clearly, cluster separation is not due to the wild or cultivated status of the variety.

It is interesting to note that fresh mass differences were most important in determining clusters for both experiments while dry mass, the usual biomass parameter, was not. Cluster groups had a clear positive or negative mean mycorrhizal effect size for fresh mass in both cluster analyses, while the other determining factors had more of a gradient response. In the first experiment, the mycorrhizal effect on acid phosphatase level, root diameter and % of small roots were also statistically important in distinguishing clusters. Root length was found to be the only other important factor for cluster determination in the second experiment; both clusters had increased root length with mycorrhizae. In this study, mass was the most important determinant of MD based on effect size, while root architecture was also found to be important.

Other research has shown that MD is closely related to mass differences, root architecture, and P responsiveness (Hetrick et al., 1992 and 1996). Increased shoot mass, acid phosphatase and root diameter, as well as R/S decreases were further pinpointed as determinants of MD in soja (Khalil et al., 1994 and 1999). It has also been suggested that C drain might be more important in determining biomass increases, and therefore MD, than mineral uptake (Haynes et al., 1991; Hetrick et al., 1996). The results of the present study generally correspond with the findings of other researchers. The R/S was found to correspond well to MD in the second experiment of this study, varieties with higher R/S having higher MD. Our attempt to assess MD by amalgamating the effect of mycorrhizae

on many different parameters simultaneously, found fewer factors to be important than the literature would suggest because so few factors significantly affected cluster makeup.

Despite the small number of factors influencing clustering, some trends can be noted by observing the data patterns in the second experiment. Forestburg and ONP are most MD for the physiological factors; Forestburg and the wild varieties have the most root architecture characteristics that indicate high MD; ONP, Caddo and Forestburg responded best to mycorrhizae for mineral uptake. Overall, the cultivated group tends to respond more dramatically to mycorrhizae, mostly based on the influence of Forestburg, the cultivar that has not undergone agricultural selection. The genetic variety of switchgrass was the most important determinant of MD in this study. These experiments have shown that varieties respond to mycorrhizae by altering different parameters. It was also noted that by changing one growth condition, soil volume, mycorrhizal responsiveness differed. This suggests that the specific environment does mediate response.

2.7- Wild vs. Cultivated

The varieties in this study have shown distinct responsiveness patterns to mycorrhizal colonization. Because of the strong responses of some varieties for some of the parameters, most of the comparisons between wild and cultivated groups suggest that the cultivated group is the more mycorrhizal responsive group. However, this trend is most likely due to the positive responses to mycorrhizae that were seen in the Forestburg variety. Since Forestburg is a cultivar that has not undergone selection under an

agricultural environment, it is reasonable that in the cluster analysis, it groups with the wild varieties more closely than with the other cultivars.

The highly selected varieties NU and Caddo have different responses to mycorrhizae. NU does not respond consistently with either positive or negative effects. In the first experiment its responses are more positive, and in the second they are neutral. It is not clear whether selection under an agricultural environment has affected the MD of the variety NU. Caddo does not respond to mycorrhizae by altering its physiological or root parameters, but does show a positive response in terms of mineral uptake. Mycorrhizal functioning as determined by ^{32} P uptake was also found not to be related to biomass increases in wheat cultivars (Hetrick et al., 1996). It could be that the mineral efficiency, i.e. the ability to use minerals to increase biomass or fitness, is low in this switchgrass cultivar. The high level of acid phosphatase in the cultivar Caddo also suggests low P efficiency (McLauchlan, 1980). The overall mineral levels were lower in Caddo, possibly allowing for low mineral efficiency. It is possible that mycorrhizae are benefiting Caddo plants, but the poor mineral conversion efficiency lowers its mycorrhizal dependency. In this case I would conjecture that MD is decreasing for Caddo.

The response of the wild switchgrass varieties is puzzling in terms of expected results. Previous research on wild populations of switchgrass has indicated high dependency on mycorrhizae for growth (Hetrick et al., 1988; Bredja et al., 1998; Entry et al., 1999). In this study, mycorrhizal colonization was detrimental for Ojibway in almost all categories, while PH had unclear responses, some positive and some negative. The only wild variety

that shows high MD is ONP. Because of this, we cannot affirm that the natural condition of switchgrass is to have a high MD. However, since the studies mentioned above took place in the U.S mid west, the place of origin for the switchgrass populations that were made into agricultural cultivars, their comparisons of wild and cultivated plants may be less tainted by the possibility of geographic ecotype differences. It has been shown that the shorter growing season found in the colder climate of Canada limits sporulation of *Glomales* in association with warm season grasses; this may induce the symbiosis to be less beneficial during colder times of the year (Bentivenga and Hetrick, 1991). The effect of climate may be limiting the MD of wild switchgrass in Canada.

Based on mass and root architecture, cultivated wheat was shown to be more MD than its wild ancestors in several studies (Kapulnik and Kushnir, 1991; Hetrick et al., 1992; Hetrick et al., 1993). In highly cultivated species such as wheat, corn and soja, the responses of the less selected varieties were not consistent with each other. This corresponds to what was found for wild switchgrass varieties in this study. For soybean plants, higher MD was based on root architecture, increases in P and acid phosphatase (Khalil et al., 1999). Although mass responses were higher in certain non-selected varieties of soja and corn, mineral responses were in general greater in the selected cultivars. These results led the authors to conclude that although cultivars were not more MD than non-selected varieties, the latter were quite different from each other in their responses to mycorrhizae (Khalil et al., 1994).

Wild oats, which were less MD than cultivars based on mass, were observed to have higher R/S, %N, and P efficiency, as well as slower growth (Haynes et al., 1991). Cultivated tomatoes were determined to be more MD based on higher % colonization, mass increases, P content and seed number increases, while root plasticity was greater and days to flowering was shorter in mycorrhizal wild varieties (Bryla and Koide, 1990a & b). The wild switchgrass in this study was similar to the wild varieties in those studies as they all have higher R/S, %N, slower growth and more root plasticity than cultivars. Forestburg, the variety with the highest MD, has a high R/S but fast growth and low %N, in contrast to the wild types. These traits of wild switchgrass are likely adapted to deal with unfavourable soil conditions. Since cultivars are less innately prepared to cope with adverse conditions, based on root morphology and growth rate, cultivars should be either more MD, which contradicts predictions from cost/benefit analyses, or did not encounter environmental stress during selection. It is possible that successful non-selected cultivars, such as Forestburg, have both high mycorrhizal responsiveness and large root systems as a functional redundancy, and this is one of the reasons why they grow successfully in marginal conditions.

Corn, soja and wheat are 'high input' crops which receive lots of fertilizer and pesticides when grown under agricultural conditions. Less fertilizer or chemicals are applied to oats and possibly tomatoes. The lower input crops (oats, tomato) have been found to have an increased MD in cultivation, while wild varieties are more MD for corn, soja and wheat. Switchgrass, as a low input crop, is more likely to respond similarly to oats than to wheat.

2.8- Implications for Agriculture

The inclusion of mycorrhizae in sustainable agriculture has been a vision to many mycorrhizal researchers (Hayman, 1982; Hamel, 1995; Plenchette and Strullu, 1995). Selective breeding for MD may have a place in sustainable agriculture practices. This study has shown that there are variations in the MD of switchgrass varieties, and that the manner by which cultivars are affected by mycorrhizae depends on variety and environment. For example, based on the results of this study, if the goal was to maximize biomass production with mycorrhizae, Forestburg or ONP would be the best varieties. If the goal were to grow on low mineral soil, Caddo or ONP, which gain the most by mycorrhizae in terms of mineral content and have low mineral requirements, would be more appropriate. The genetic diversity among switchgrass varieties remains high and therefore selective breeding for MD based on the variances already seen in varieties is feasible. It should be noted however, that plant-fungal species preference might be important in determining response of switchgrass to mycorrhizal fungi.

Farming practices that are less likely to disrupt natural mycorrhizal populations are being promoted as part of a sustainable agriculture management plan. In Europe, where organic farming is more widespread and there is some aversion to using genetically modified organisms, mycorrhizae-friendly farming practices are more likely to take hold. Organic farms which did not till and used crop rotation, experienced a higher than expected yield, only 10% less than conventional systems, and this was attributed to mycorrhizae (Mader et al., 2000).

Low-input farming has the additional advantage of maintaining natural levels of nutrients, mycorrhizal populations, and biodiversity in the soil (Podesfinski et al., 2001). Under these conditions, inadvertent selection against mycorrhizal symbiosis should not occur. Lower MD of cultivated wheat and lower MD of corn selected for fungal resistance, indicate that selection against mycorrhizae does occur in agricultural systems. The fungi themselves are also subject to inadvertent selection. It has been suggested that AM fungal species that can colonize new roots from colonized root particles in the soil or mycelium broken off from the network are at a selective advantage in tilled systems. *Glomus* species can colonize from spores, hyphae or infected roots while *Gigaspora* species depend more on their large spores for propagation (Smith and Read, 1997). Pot experiments by Boddington and Dodd (2000b) led them to suggest that *Glomus* species are 'aggressive' and selected for under agricultural environments while *Gigaspora* species are not. A similar effect was found by Hendrix et al. (1995) where *Gigaspora* species dominated under a monoculture of soybean, while *Glomus* species dominated under crop rotations.

While some *Glomus* species have been shown to be compatible symbiotic partners for switchgrass (Clark and Zeto, 1999; Eom et al., 2000), *Gigaspora* species are more common in some agricultural soils (Podeszfinski et al., 2001) and in mature tallgrass prairie soils (R.M Miller, pers. comm.). The possible inadvertent selection under conventional agricultural systems for certain mycorrhizal species may limit the responsiveness of switchgrass, since symbiont preference has been shown in this species. This is another argument in favour of non-disruptive practices for sustainable agriculture.

3.0- Conclusions

As a general rule, we cannot conclude that the wild varieties of switchgrass are more MD than cultivars, as was stated in our original hypothesis. The present study concurred with other studies on cultivation and mycorrhizae, which found that wild varieties are inconsistent in their MD. Therefore, it is not justified to compare wild varieties to cultivars as a unit. In the cluster analysis of the second experiment, the cluster of ONP, PH and Forestburg encompasses the varieties that responded positively to mycorrhizal colonization for most of the parameters. Among these, the first two are wild and the third one is cultivated but has not undergone any selection. NU and Caddo, the selected cultivars, clustered together and with the wild variety Ojibway. Varieties in this group tended to have neutral or negative responses to mycorrhizae, but not for every parameter.

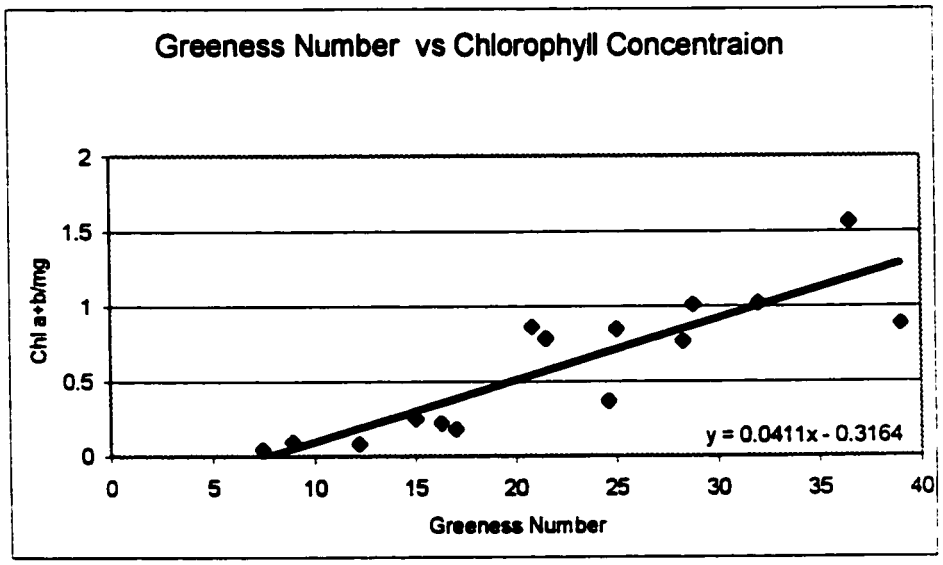
The most important factors in determining mycorrhizal response were the variety of switchgrass and the growth conditions, especially the soil volume, not the cultivation level. It was noted that not only do varieties differ in MD, but in the type of response they have when mycorrhizal. Varieties respond with either increased physiological, root or mineral parameter responses. Mineral increases did not correlate with biomass increases in this study. Assessment of MD by CDA on effect size found in both experiments that fresh mass was the most important parameter by which varieties were clustered. It was also observed the R/S was a good predictor of MD in the second experiment. These results are in agreement with other studies, which determined that biomass increases and root architecture are important determinants of MD in grasses.

There are definite differences in MD among switchgrass varieties in this study. Plant breeders may enhance these genotype differences to increase the MD of certain cultivars. A cultivar that is highly mycorrhizal efficient would be attractive for sustainable agriculture practices.

To further determine the impact of agriculture on the ability of crop plants to benefit from mycorrhizal associations, similar studies should be done for other species for which a breeding history record exists. It might also be prudent to use species that have been cultivated for longer than switchgrass. It would also be interesting to pursue the nature of the MD differences found among the switchgrass varieties used in this study. A field study would help to determine if mycorrhizal responsiveness differs under a more natural environment. An examination of the natural soil types and fungal populations at the wild sites might indicate why wild varieties ONP and Ojibway showed opposite mycorrhizal responses despite being geographically close. The question of switchgrass ploidy remains elusive. It is recommended that flow cytometry be used to determine switchgrass ploidies. This technique would be useful to compare cultivar ploidies to those found by other researchers, and it would be both interesting and important to assess the ploidy of wild Canadian populations of switchgrass for prairie revegetation efforts.

Appendix

1.0- Standard curve for greenness number vs. chlorophyll concentration.



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