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# ARBUSCULAR MYCORRHIZAL FUNGAL COLONIZATION IMPROVES REGROWTH OF BERMUDAGRASS (*CYNODON DACTYLON* L.) AFTER CUTTING

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**Abstract**

The interations of plant regrowth, non-structural carbonhydrate (total soluble carbohydrate, glucose, surcose, fructose and starch) concentrations and arbuscular mycorrhizal fungi (AMF) was examined in Bermudagrass (*Cynodon dactylon* (L.) Pers. cv. Banana) at 0, 1, 2, 4, 6, 12, 18, 24 days after cutting. AMF colonization of Bermudagrass was significantly increased; aboveground dry weight of Bermudagrass colonized by AMF were significantly higher than that of non-mycorrhizal plants; the maximum of chlorophyll content of AMF colonized Bermudagrass was higher relative to non-mycorrhizal plants. The total concentrations of carbohydrate, fructose and starch in the roots of myccohizal plants were significantly lower than those in control plants, while the concentration of glucose in the roots of mycorrhizal plants was significantly higher than those of non-mycorrhizal plants. This study indicates that stimulation of plant regrowth by AMF may partly be attributed to stimulation of photosynthesis capability, and that glucose may be the main form of carbohydrate, which AMF absorb from their grass host.

## Introduction

The roots of most higher plants live in symbiosis with arbuscular mycorrhizal fungi (AMF) (Li & Feng, 2001). AMF are obligate biotrophic fungi that can improve the mineral nutrition of host plants, but they depend on the supply with carbohydrates by the host plant (Smith & Read, 1997). Other potential benefits of AMF colonization include: increased tolerance of roots to soil-borne pathogens (Gianinazzi-Pearson & Gianinazzi, 1983; Heald *et al*., 1989), improved drought stress (Davies *et al.*, 1992), and increased protection from salt stress (Rosendahl & Rosendahl, 1991). In short, AMF improve plant mineral nutrition and protect them against environmental stress. Most species of turfgrass in their undisturbed environments form a beneficial association with mycorrhizal fungi. The AM symbiosis, once established on the turf root system, radiate out from the roots to form a dense network that improves the uptake of poorly mobile nutrient ions and water, enhancing plant tolerance to (biotic and abiotic) stress (Gemma & Koske, 1989; Sylvia & Burks, 1988; Hall *et al*., 1984).

Bermudagrass is an important turfgrass widely used in playgrounds and greenbelt areas. Cutting is a common way of maintaining turfgrass. Reduction in leaf area by grazing can decrease the carbon invested in roots (Richards, 1984; Trent *et al*., 1987). Such a decrease may influence AMF root colonization (Reece & Bonham, 1978; Wallance, 1981), since the fungi require plant carbohydrates as an energy source (Bjorkman, 1942; Paul & Kucey, 1981). It is of interest, however, to investigate whether under a stress treatment that limits photosynthesis, such as cutting, the plant can still compensate for the presence of the mycorrhizal fungus.

While the effect of grazing on AMF colonization or the pool of soluble carbohydrate has been studied extensively (Reece & Bonham, 1978; Wallance, 1981; Richards, 1984; Benthlenfalvay *et al*., 1985), the relationship between AMF colonization and carbohydrates under cutting condition have received little attention. Unfortunately, to our knowledge, early works relating plant carbohydrate — AMF relations only concerned the total soluble carbohydrate concentration after cutting (Trent *et al*., 1987); no study has ever been attempted to demonstrate these relations between non-structural carbohydrates and AMF colonization under an experimental treatment of cutting.

The objectives of this research were: (i) to investigate if cutting influences AMF colonization and the concentrations of non-structural carbohydrates (total soluble carbohydrate, glucose, sucrose, fructose and starch) and (ii) to study the relationships among regrowth, AM colonization and non-structural carbohydrate concentrations.

## Materials and Methods

**Experimental design:** An experiment was conducted at Sun Yat-sen University (23°10.126´N, 113°21.717´E) Guangzhou, China, from August 2006 to November 2006. Soil was collected from Bamboo Park of Sun Yat-sen University. A mixture of sand and soil (1:1 vol $vol^{-1}$), was sieved through a 2 mm mesh, autoclaved to eliminate indigenous AMF. The soil mixture had the following properties: pH 6.7 (soil: water ratio, 1: 2.5), organic matter = 1.28%, nitrogen = 41.97 mg $kg^{-1}$, phosphorus = 56.05 mg $kg^{-1}$ and potassium = 62.42 mg $kg^{-1}$. 3 kg dry weights of the soil mixture were filled into each round plastic pot (diameter: 21 cm; height: 12.6 cm). Initially, compound fertilizer (N: P: K=15: 15: 15) 0.5 g $kg^{-1}$ was added to each pot.

Inoculum of the AMF, *Glomus intraradices* (Schenck & Smith), was comprised of a mixture of sandy soil containing dried roots, hyphae and spores. The isolate BGCUSA05 of *G. intraradices* was provided by Dr Wang, YS from Beijing Academy of Agriculture and Forestry Science, China. Inoculum (30 g) was mixed with the sandy soil of each pot for the mycorrhizal treatment prior to planting. Bermudagrass (*Cynodon dactylon* (L.) Pers. cv. Banana) was studied. Seeds were sown at 0.5 g $pot^{-1}$ at a depth of 1.5 cm. The experimental pots were placed on the top of a six-floor high building under natural light and temperature. Water was added every two days during the first week and once per day afterwards.

To study the relationship of carbohydrate concentration and AMF colonization in turfgrass after cutting, a factorial design was used. It consisted of a mycorrhizal inoculation treatment, inoculation with *G. intraradices* versus non-mycorrhizal control, and eight sampling dates (0, 1, 2, 4, 6, 12, 18, 24 d after cutting). 71 days after seeding, the shoots were cut, 1 cm aboveground. Three replicate pots of mycorrhizal and non-mycorrhizal plants (total 48 pots) were harvested eight times at 0, 1, 2, 4, 6, 12, 18, 24 d after cutting.

**Evaluation of mycorrhizal colonization:** After each harvest date, random samples of fresh roots were excised and microscopically evaluated for mycorrhizal colonization. The level of mycorrhizal colonization was determined using the line intersection method (McGonigle *et al*., 1990) after staining the fungal structures with Trypan Blue (Philips & Hayman, 1970). Root samples were cut into 1-cm-long segments, cleared in 10% (w/v) KOH in a water bath at 90°C for 40 min, acidified in 2% (v/v) HCl and then stained with Trypan Blue at 90°C for 40 min. Forty root segments from each subsample were observed under the microscope for colonization.

**Plant growth measurements:** Plant growth was assessed in terms of total plant dry weight (DW). Fresh shoots and roots were separated, and half was taken for measurements of mycorrhizal root colonization and chlorophyll content and the other half dried at 70°C for 48 h. Then they were weighed and used for determining the carbohydrate concentrations.

**Analytical methods for carbohydrate concentration:** The shoots and roots were dried at 70°C for 48 h and analyzed for carbohydrate concentration. Total soluble carbohydrate was assayed by the methods of Fairbairn (1953); sucrose, by the methods of Li (1998); fructose, glucose and starch, by the methods of Li (2000).

**Statistical analysis:** Data were subjected to one-way ANOVA using the SPSS 10.0 software. Means were compared by the LSD test and statistical significance are reported at 5% level. Data on mycorrhizal parameters and non-structural carbohydrate concentration were subject to Pearson Correlation analysis.

## Results

**AMF colonization:** Total, hyphal and vesicle colonization of AMF-inoculated Bermudagrass roots increased significantly ($p<0.05$) over time (Fig. 1). No arbuscules were found in the pot culture experiment. No colonization by AMF was found in the non-inoculated control plants.

**Plant regrowth:** Fig. 2 shows the effect of cutting on biomass of AM-inoculated and non-inoculated Bermudagrass. The aboveground biomass of the mycorrhizal plants was significantly ($p<0.05$) higher than that of the non-mycorrhizal plants up to 12 days after cutting. Root biomass decreased significantly until 12 days after cutting, and then it started to recover, showing no differences between mycorrhizal and non-mycorrhizal plants. Similar with underground biomass, total biomass decreased significantly until 12 days after cutting, and then started to recover. And total biomass of mycorrhizal plants were higher than those of non-mycorrhizal plants during 16 days after cutting. The shoot biomass correlated significantly with total colonization, the root biomass showed a significant negative correlation with vesicle and total colonization (Table 1).

The content of chlorophyll of the AM grass was higher than that of the non-mycorrhizal grass 5 days after cutting. And mycorrhizal plants reached their maximum of chlorophyll content 6 days earlier than non-mycorrhizal plants (Fig. 3).

**Non-structural carbohydrates:** The non-structural carbohydrate contents are showed in Fig. 4. Unlike the concentration of the other carbohydrates that of glucose was significantly higher in shoots and roots of AMF inoculated than non-inoculated Bernmudagrass plants (Fig. 4. d, i). This was particularly true for the concentration of glucose in the shoots, which was more than twice as high in AM than non-mycorrhizal plants. The starch concentrations in shoots and roots of AMF-inoculated Bermudagrass were significantly ($p<0.05$) lower than those of non-inoculated plants (Fig. 4. e, j). The concentration of total soluble carbohydrate, sucrose and fructose in roots of AMF-inoculated Bermudagrass dropped to the minimum at day 6 after cutting, and then increased again, while the concentrations of sucrose and fructose in roots of non-inoculated plants dropped to their lowest levels at day 2 after cutting and recovered afterwards (Fig. 4. f-h).

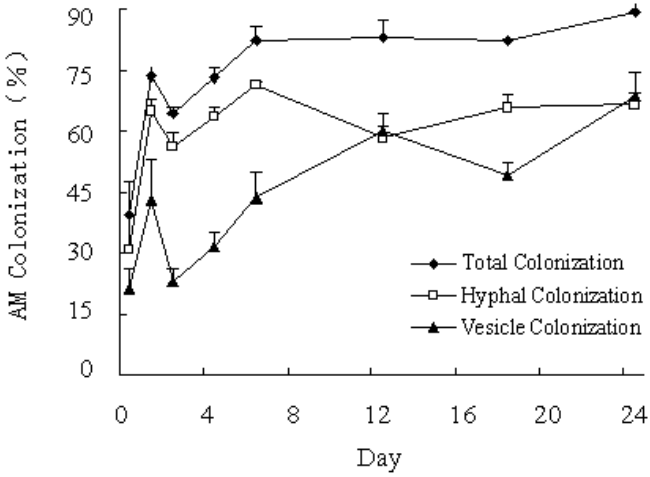


Fig. 1. AM colonization of Bermudagrass in 0-24 days after cutting.

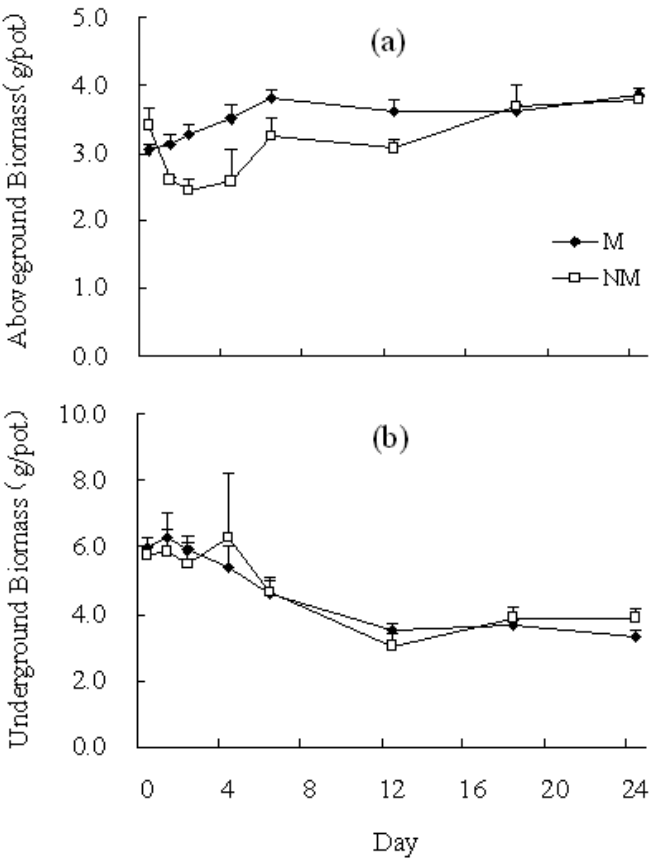


Fig. 2. Shoot (a) and root (b) dry weight of Bermudagrass (*Cynodon dactylon*) 0-24 days after cutting. M is the acronym of mycorrhizal plants; NM is the acronym of nonmycorrhizal plant.

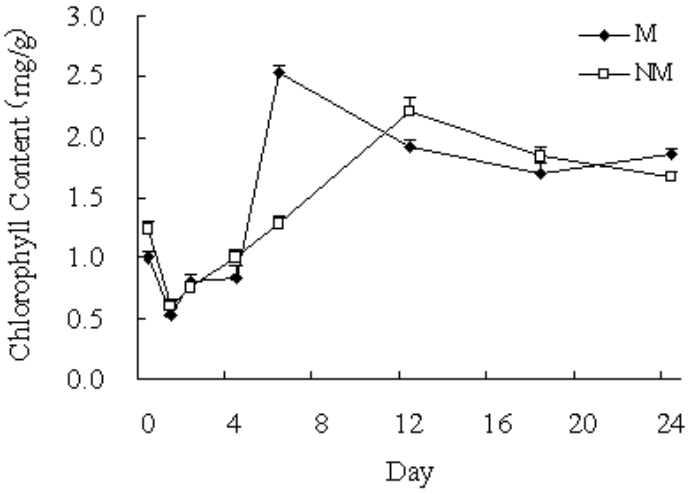


Fig. 3. Chlorophyll content of Bermudagrass in 0-24 days after cutting. M is the acronym of mycorrhizal plants; NM is the acronym of nonmycorrhizal plant.

## Discussion

**Influence of cutting on AM colonization:** Reduction in leaf area by grazing can decrease the amount of carbohydrates, which are invested in roots (Richards, 1984). Reduction of leaf area may influence AMF root colonization, since AMF require plant carbohydrates as an energy source (Paul & Kucey, 1981). It has been reported from eight grass species that AMF colonization decreased by grazing, owning to the decreased leaf area and increased root to shoot ratio (Bethlenfalvay & Dakessian, 1984). However, other studies have shown different responses of AMF colonization to grazing. Wallace (1981) found decreased AMF colonization after grazing, while Reece & Bonham (1978) detected no effects of grazing on AMF colonization. In the present study, AM colonization was increased after cutting in Bermudagrass (Fig. 1). Although the reason is unclear, we propose that the effect of cutting on AMF colonization is plant species dependent.

**Mycorrhiza favors plants regrowth:** Many researchers have reported enhanced regrowth of mycorrhizal versus non-mycorrhizal plants (Koide & Mosse, 2004; Borkowska, 2002; Henrike *et al*., 2007; Kaya *et al*., 2003). AMF colonization generally increases plant growth comparing to non-mycorrhizal plants (Trimble & Knowles, 1995). In the present study, the aboveground biomass of mycorrhizal Bermudagrass was significantly greater than that of non-mycorrhizal plants (Fig. 2). We propose that AMF inoculation can improve regrowth of host plants after cutting. Growth stimulation by AMF is generally explained by increased nutrient absorption, especially phosphorus (Kaya *et al*., 2003; Koide & Mosse, 2004; Henrike *et al*., 2007). Some researchers also reported the improved photosynthetic rate by AMF inoculation (Borkowska, 2002; Fan *et al*., 2008; Sheng *et al*., 2008). In our study, we found higher chlorophyll contents in mycorrhizal plants (Fig. 3), indicating that AMF, indeed, may stimulate photosynthesis. Although the mechanism has not yet been determined, the improved regrowth after AMF inoculation could partly be ascribed to a higher photosynthetic rate in mycorrhizal plants.

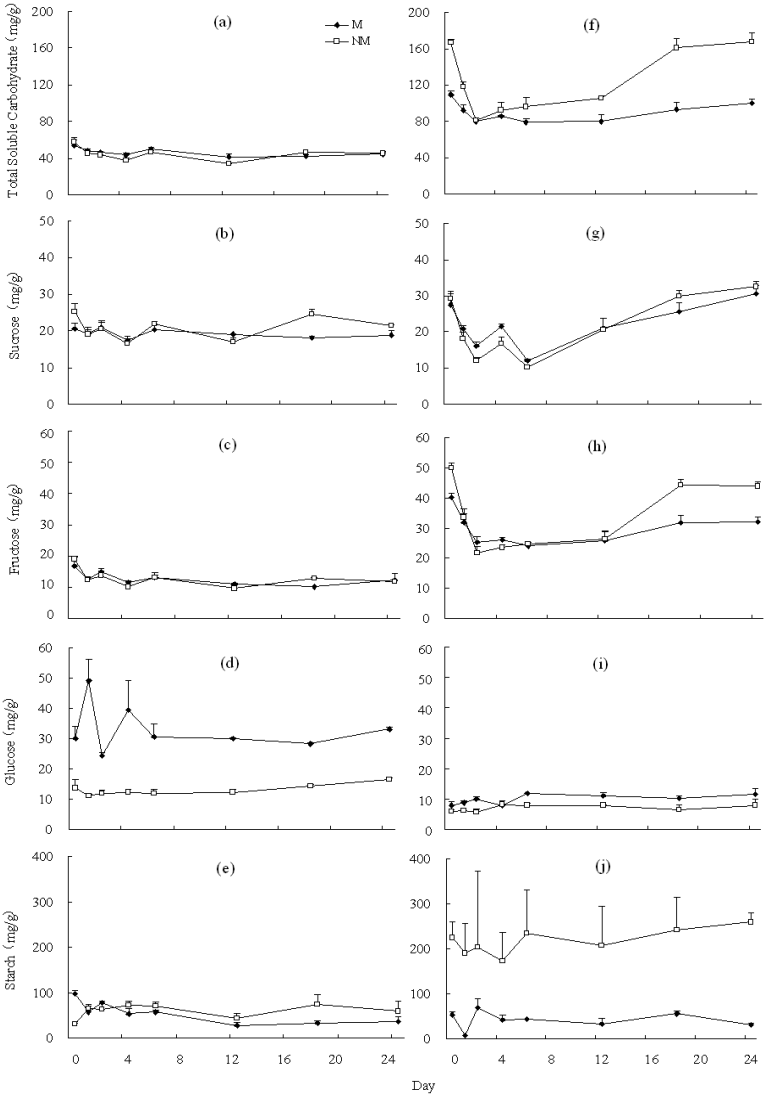


Fig. 4. Concentrations of total soluble carbohydrate (a), surcrose (b), fructose (c), glucose (d) and starch (e) in shoots (dry-weight basis) and concentrations of total soluble carbohydrate (f), surcrose (g), fructose (h), glucose (i) and starch (j) in roots (dry-weight basis) of nonmycorrhizal and mycorrhizal Bermudagrass in 0-24 days after cutting. M is the acronym of mycorrhizal plants; NM is the acronym of nonmycorrhizal plant.

**Table 1. Correlations between total colonization, hyphal colonization, vesicle colonization and biomass of Bermudagrass (n=24).**

| | **Total colonization (%)** | **Hyphal colonization (%)** | **Vesicle colonization ( %)** |
|---|---|---|---|
| **Shoots biomass (g)** | 0.449* | 0.363 | 0.398 |
| **Roots biomass (g)** | -0.658** | -0.410* | -0.719** |

* and ** represent significance at the level of 0.05 and 0.01, respectively

**Response of the non-structure carbohydrates to AMF colonization:** Generally, starch concentration was very low in AMF colonized plants, and that was relevant to the hepatin accumulation (Liu *et al*., 2007). It is the same with starch in our experiment. The starch concentrations in shoots and roots of AM-infected plants were lower than those in non-mycorrhizal plants. This may be because starch may have been hydrolyzed to soluble carbohydrate in AM-infected plants.

Our results also support the hypothesis that glucose may be the main form of carbohydrate that AMF fungi absorb from host plants (Bago *et al*., 1999; Pfeffer *et al*., 1999). Unlike other non-structure carbohydrates, the glucose concentration in shoots and roots of AMF colonized Bermudagrass were higher than those in non-mycorrhizal plants. However, the dynamics in the total non-structural carbohydrate content after cutting is dominated by the fructose and sucrose concentrations not by those of glucose and starch. Therefore, there was non direct correlation between the AMF root colonization levels and the concentrations of these carbohydrates. Additionally, AM colonization increased plant photosynthetic rate relative to non-mycorrhizal plants, which may have led to higher glucose concentrations in mycorrhizal plants.

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