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Effects of arbuscular mycorrhizal fungi on photosynthesis, carbon content, and calorific value of black locust seedlings

X.Q. ZHU*, C.Y. WANG**, H. CHEN**, and M. TANG**, +

College of Life Sciences, College of Forestry**, Northwest A&F University, Yangling, Shaanxi 712100, China*

Abstract

Arbuscular mycorrhizal fungi (AMF) form symbioses with many plants. Black locust (*Robinia pseudoacacia* L.) is an important energy tree species that can associate with AMF. We investigated the effects of AMF (*Rhizophagus irregularis* and *Glomus versiforme*) on the growth, gas exchange, chlorophyll (Chl) fluorescence, carbon content, and calorific value of black locust seedlings in the greenhouse. The total biomass of the arbuscular mycorrhizal (AM) seedlings was 4 times greater than that of the nonmycorrhizal (NM) seedlings. AMF greatly promoted the photosynthesis of black locust seedlings. AM seedlings had a significantly greater leaf area, higher carboxylation efficiency, Chl content, and net photosynthetic rate (P_N) than NM seedlings. AMF also significantly increased the effective photochemical efficiency of PSII and significantly enhanced the carbon content and calorific value of black locust seedlings. Seedlings inoculated with *G. versiforme* had the largest leaf area and highest biomass, Chl content, P_N, and calorific value.

Additional key words: chlorophyll fluorescence; gas exchange; growth; symbiosis.

Introduction

Black locust (*Robinia pseudoacacia* L.) is a fast-growing tree species that is native to the south-eastern USA; however, it has been widely planted in temperate terrestrial ecosystems around the world (Barrett *et al.* 1990). This tree is often managed by silvicultural system and shows a high resistance to abiotic stresses. Nitrogen-fixing bacteria, which form nodules on its root system, enable black locust to grow on poor soils (González-García *et al.* 2011). Black locust has attracted special attention in recent years as a potential energy crop because of its high biomass yield (Gasol *et al.* 2010) and heat energy (Carpenter and Eigel 1979, Tian *et al.* 2003). For example, black locust produced 3–10 t ha^{-1} of dry biomass every year, which was much more than that produced by poplar (*Populus*) and willow (*Salix* spp.) at the same post-mining site (Bongarten *et al.* 1992, Gruenewald *et al.* 2007, Grünewald *et al.* 2009). Black locust wood can be used as firewood or it can be pyrolyzed into gas (Geyer and Walawender 1994), oil (Balat 2010) or ethanol (González-García *et al.* 2011, 2012). To generate more energy from black locust feedstock, the key issue is how to increase its biomass yield and calorific value.

The amount of biomass produced by plants is intimately related to photosynthesis. In the photosynthetic process, plants absorb solar energy and assimilate CO_2 and the products of carbon fixation are then further converted into organic materials, which are stored as the plant biomass (Zhu *et al.* 2008). Photosynthesis influences the quality of energy plants, which is measured by the carbon content and calorific value (Kumar *et al.* 2011). Calorific value is the energy produced from 1 g of fuel or by the complete combustion of the material. It can be used to reflect the physiological and growth status of plants (Ren *et al.* 1999), and is considered to be an important parameter for assessing energy plants (Kumar *et al.* 2011).

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+Corresponding author; phone: +86-29-87080157, fax: +86-29-87080157, e-mail: tangm@nwsuaf.edu.cn

Abbreviations: AMF – arbuscular mycorrhizal fungi; AM – arbuscular mycorrhizal; *CE* – carboxylation efficiency; Chl – chlorophyll; C_i – intercellular CO_2 concentration; *E* – transpiration rate; F_0 – minimal fluorescence in the dark-adapted state; F_m – maximal fluorescence in the dark-adapted state; F_0' – minimal fluorescence in the light-adapted state; F_m' – maximal fluorescence in the light-adapted state; F_s – steady-state fluorescence; F_v/F_m – maximal quantum yield of PSII in the dark-adapted state; F_v'/F_m' – maximal quantum yield of PSII in the light-adapted state; g_s – stomatal conductance; LDM – leaf dry mass; NM – nonmycorrhizal; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; q_P – photochemical quenching coefficient; (S)LA – (specific) leaf area; S/R – shoot:root ratio; RDM – root dry mass; SDM – stem dry mass; TDM – total dry mass; Φ_{PSII} – effective quantum yield of PSII.

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Many soil microorganisms greatly affect the growth status and physiology of plants. However, there are no reports to date about whether microorganisms influence the plant calorific value. If symbiotic microorganisms can improve the calorific value of the host, this would have important implications for plant energy production. Arbuscular mycorrhizal fungi (AMF) occur in almost all terrestrial ecosystems, forming symbioses with about 80% of all plant species (Smith and Read 2008). They enhance the growth and the photosynthetic ability of the host plants by improving nutrient uptake and CO_2 assimilation (Smith and Read 2008, Sheng *et al*. 2008, Kaschuk *et al*. 2009). Many studies have confirmed that AMF can form a symbiotic relationship with black locust and significantly improve its growth (Olesniewicz and Thomas 1999, Tian *et al*. 2003). However, there is little information about the effects of AMF on the photosynthesis, carbon content, and calorific value of black locust.

The goal of this study was to determine the role of AMF in black locust growth and physiology, including the effect of AMF on parameters of gas exchange, Chl fluorescence, carbon content, and calorific value. The main objectives were: (*1*) to reveal the effects of AMF on these parameters; (*2*) to compare two different AMF species, *R. irregularis* and *G. versiforme*.

Materials and methods

Soil and plant treatment: The soil (pH 7.5) used as the culture substrate for the plants was collected from the top 0–20 cm of soil layer in the campus of Northwest A&F University (Yangling, Shaanxi Province, China). It was comprised of 17.56 g kg^{-1} of organic matter, 55 mg kg^{-1} of available nitrogen, 19 mg kg^{-1} of available phosphorus, and 232 mg kg^{-1} of available potassium determined by Bao (2000). The collected soil was ground, sieved through a 2-mm sieve, mixed with fine sand [sand/soil (v/v) = 1:1] and then autoclaved at 121°C for 2 h.

Black locust seeds were collected in October 2010 from Northwest A&F University (Yangling, Shaanxi Province, China). In May 2011, the fully developed seeds were disinfected with 1% $KMnO_4$ for 10 min, and then rinsed 5 times with sterile distilled water. Then the seeds were arranged on sterilized moist paper to germinate at 25°C in a plant growth chamber. Initially 3 pregerminated seeds were sown in each pot (top diameter of 17 cm; depth of 16 cm) containing 1.5 kg of culture substrate. Seedlings were thinned to 1 seedling per pot 10 d after sowing.

AMF inoculation and experimental design: The two AMF inocula, *R. irregularis* (Bank of Glomales in China, No. BGC BJ09) (synonym for *Glomus intraradices* DAOM 197198) (Krüger *et al*. 2012) and *G. versiforme* (Karsten) Berch (No. BGC GD01C), were provided by the Bank of Glomales in China (BGC). The mycorrhizal inocula were comprised of spores (about 50 spores g^{-1}), hyphae, infected root fragments, and soil. Each pot receiving an arbuscular mycorrhizal treatment (AM) was inoculated with 20 g of either *R. irregularis* or *G. versiforme* inocula and sown with pregerminated seeds. The nonmycorrhizal (NM) pots received the treatment comprising of 20 g sterilized inocula with 10 ml of fungi-free filtrate from the living inocula suspension and they were sown with pregerminated seeds. There were 5 replicates of each treatment. The pots were randomly arranged in a greenhouse under natural light, at a temperature of 12–35°C and humidity of 40–85%. Each pot was supplemented weekly with 100 ml of half-strength Hoagland nutrient solution and with 200 ml of water every 3 d. The experiment lasted from May to October 2011.

Growth parameters: Five months after sowing, the stem heights and basal diameters (stem diameters at 1 cm above the soil surface) of the seedlings were measured. Five seedlings per treatment were harvested and the following parameters were assayed. Leaf area (LA) was determined using the disc method described by Gao (2006): 30 leaf discs (1.66 cm^2 per disc) were cut from the 3rd, 4th, and 5th fully expanded, compound leaves (from the top) and then dried to obtain their dry mass. The LA (cm^2) of the whole seedling was then calculated by determining the leaf dry mass (LDM) of the whole seedling specific leaf area (SLA, cm^2 g^{-1}). Root dry mass (RDM), stem dry mass (SDM), and LDM were determined by drying the roots, stems, and leaves to constant mass in an oven at 80°C. LA, total dry mass (TDM) and shoot:root (S/R) ratios were calculated as follows:

$$LA = LDM \times SLA$$
$$DM = RDM + SDM + LDM$$
$$S/R = (SDM + LDM)/RDM$$

AMF colonization measurement: To measure the AMF colonization rate, about 1 g of fresh roots were washed first gently under running tap water, and then rinsed 3 times with distilled water. Then, the roots were cleared in 10% KOH at 90°C for 20 min, bleached in alkaline H_2O_2 for 20 min, acidified in 1% HCl, rinsed 3 times in distilled water, and then stained with 0.05% (w/v) Trypan Blue (Phillips and Hayman 1970). Finally, the AMF coloniation rate was measured by the grid line intersection method, *i.e.*, by counting the number of colonized roots at root-grid intersection points using grid lines drawn on a Petri dish (Giovannetti and Mosse 1980, Sun and Tang 2012).

Chl content and gas-exchange measurements: The terminal leaflet of the 4th, fully expanded, compound leaf (from the top) of each black locust seedling was used to assay Chl content, gas exchange, and Chl fluorescence parameters. Chl content was measured by using a SPAD Chl meter (*SPAD-502*, *Konica Minolta Sensing*, *Inc.*, Japan) according to the manufacturer's instruction. Gas-exchange parameters, including the net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), and the transpiration rate (E), were measured by using a portable open flow gas-exchange system *LI-6400* (*LI-6400*, *LI-COR*, Lincoln, NE, USA) from 08:30 to 11:30 in the morning. The photosynthetically active irradiation was 1,200 μmol m^{-2} s^{-1}, CO_2 concentration was 400 cm^3 m^{-3}, the leaf temperature was 25°C, and the air flow rate was 0.5 dm^3 min^{-1}. Carboxylation efficiency (CE) was calculated as: $CE = P_N/C_i$

Chl fluorescence parameters were measured using a *MINI-Imaging-PAM* (*Walz*, Germany) at room temperature. The leaves were placed in darkness for 30 min and then the minimal fluorescence in the dark-adapted state (F_0) was recorded. Next, a saturating pulse of irradiation 2,000 μmol m^{-2} s^{-1} was administered for 3 s to determine the maximal fluorescence (F_m) in the dark-adapted state (Gong *et al.* 2013). The leaves were then placed under actinic light of 300 μmol m^{-2} s^{-1} to determine the maximal fluorescence (F_m') and the minimal fluorescence in the light-adapted state (F_0'), and the steady-state fluorescence (F_s). Photochemical quenching coefficient (q_P), nonphotochemical quenching coefficient (NPQ), the maximal quantum yield of PSII in the dark-adapted state (F_v/F_m) or in the light-adapted state (F_v'/F_m'), and effective photochemical efficiency of PSII (Φ_{PSII}) were calculated according to the following equations (Genty *et al.* 1989, Zai *et al.* 2012):

$$F_v/F_m = (F_m - F_0)/F_m$$
$$F_v'/F_m' = (F_m' - F_0')/F_m'$$
$$q_P = (F_m' - F_s)/(F_m' - F_0')$$
$$NPQ = (F_m - F_m')/F_m'$$
$$\Phi_{PSII} = (F_m' - F_s)/F_m'$$

Carbon content and calorific value measurements: The dry roots, stems, and leaves were ground to a fine powder that could pass through a 100-mesh or a 200-mesh sieve. The carbon content of 5 mg samples of ground, dry roots, stems, and leaves were determined using a *Liqui TOCII* analyzer (*Elementar*, Germany).

The calorific values of 1 g of oven-dried powder (sieved through a 100-mesh sieve) of seedling roots, stems, and leaves were measured using an automatic isoperibol calorimeter (*OR2010*, China). Benzoic acid was used as a standard (calorific value = 26,470 ± 20 J g^{-1}).

Statistical analyses: The data were subjected to one-way analysis of variance (*ANOVA*). Means were compared by *Duncan's* test at $P<0.05$ by *SPSS 16.0* (*SPSS*, Chicago, IL, USA), $n = 5$. Figures were constructed with *Sigmaplot 12.0* (*Systat Software*, San Jose, CA, USA).

Results

AMF colonization and plant growth: Black locust seedlings were colonized in 73% by *R. irregularis* and in 66% by *G. versiforme* (Table 1). AMF colonization was not observed in the roots of the control seedlings.

AMF greatly promoted the growth of black locust seedlings and increased their biomass yield (Table 1). LA were 460% and 491% larger in AM seedlings colonized by either *R. irregularis* or *G. versiforme,* respectively, and their stem heights were 72.3% and 103% higher, respectively, than that of NM plants. The basal diameters of AM seedlings were about 56% wider than those of NM seedlings. The RDM, SDM, and LDM of the AM seedlings were significantly greater than those of the NM control. The TDM of AM seedlings colonized by either *R. irregularis* or *G. versiforme* was 311% and 353% greater, respectively, than that of the NM seedlings. *G. versiforme* had a greater influence on seedling growth than *R. irregularis*. Compared with the NM control, the S/R ratios of the *R. irregularis* and *G. versiforme* AM seedlings were 35% and 27% higher, respectively.

Table 1. Effects of *Rhizophagus irregularis* and *Glomus versiforme* on AMF colonization, leaf area, height, basal diameter, shoot:root (S/R) ratio, root dry mass (RDM), stem dry mass (SDM), leaf dry mass (LDM), and total dry mass (TDM) of black locust seedlings. Means ± SD labeled with *different letters* within each line are significantly different ($P<0.05$) using *Duncan*'s test; $n = 5$.

Parameters	*R. irregularis*	*G. versiforme*	Control
AMF colonization [%]	73 ± 3.0^a	66 ± 3.6^b	0^c
Leaf area [$cm^2 pot^{-1}$]	717 ± 11.80^b	756 ± 21.50^a	128 ± 4.19^c
Height [cm]	28.4 ± 0.7^b	32.2 ± 2.8^a	15.9 ± 1.0^c
Basal diameter [mm]	3.7 ± 0.2^a	3.8 ± 0.2^a	2.4 ± 0.2^b
S/R ratio [%]	1.99 ± 0.09^a	1.77 ± 0.09^b	1.29 ± 0.05^c
RDM [g pot^{-1}]	2.05 ± 0.08^b	2.45 ± 0.13^a	0.66 ± 0.04^c
SDM [g pot^{-1}]	1.17 ± 0.08^b	1.32 ± 0.05^a	0.33 ± 0.03^c
LDM [g pot^{-1}]	2.94 ± 0.10^a	3.02 ± 0.08^a	0.50 ± 0.02^b
TDM [g pot^{-1}]	6.17 ± 0.08^b	6.80 ± 0.07^a	1.50 ± 0.03^c

Chl content and gas exchange: The Chl content of AM plants was significantly higher than that in NM plants. No significant difference was found between both AMF. The plants inoculated with *G. versiforme*, contrary to *R. irregularis*, showed the *CE* 89% higher than that of the NM plants (Table 2).

Moreover, AMF symbiosis markedly influenced the gas exchange of plants (Table 2). Both AMF enhanced significantly the P_N of the AM seedlings. The C_i, g_s, and E of *G. versiforme* inoculated seedlings were significantly lower than those of *R. irregularis* inoculated seedlings and NM seedlings.

Table 2. Effects of *Rhizophagus irregularis* and *Glomus versiforme* on chlorophyll (Chl) content, carboxylation efficiency (*CE*), net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), and transpiration rate (*E*) of black locust seedlings. Means ± SD labeled with *different letters* within each line are significantly different ($P<0.05$) using *Duncan*'s test; $n = 5$.

Parameters	*R. irregularis*	*G. versiforme*	Control
Chl content [SPAD]	26.04 ± 3.655^{a}	29.12 ± 1.310^{a}	21.80 ± 1.298^{b}
CE [µmol (CO_2) m^{-2} s^{-1}]	0.033 ± 0.003^{b}	0.055 ± 0.003^{a}	0.029 ± 0.007^{b}
P_N [µmol (CO_2) m^{-2} s^{-1}]	8.965 ± 0.389^{a}	9.484 ± 0.741^{a}	7.931 ± 0.441^{b}
C_i [µmol (CO_2) mol^{-1}]	272.1 ± 16.82^{a}	177.4 ± 34.94^{b}	283.6 ± 54.38^{a}
g_s [mmol (H_2O) m^{-2} s^{-1}]	117.7 ± 14.9^{a}	71.5 ± 17.0^{b}	127.9 ± 13.2^{a}
E [mmol (H_2O) m^{-2} s^{-1}]	0.572 ± 0.120^{a}	0.362 ± 0.067^{b}	0.598 ± 0.198^{a}

Table 3. Effects of *Rhizophagus irregularis* and *Glomus versiforme* on chlorophyll fluorescence parameters of black locust seedlings. Means ± SD labeled with *different letters* within each line are significantly different ($P<0.05$) using *Duncan*'s test; $n = 5$. F_m – maximal fluorescence in the dark-adapted state; F_0 – minimal fluorescence in the dark-adapted state; F_m' – maximal fluorescence in the light-adapted state, F_0' – minimal fluorescence in the light-adapted state; NPQ – nonphotochenical quenching coefficient; q_P – photochemical quenching coefficient; F_v/F_m – maximal quantum yield of PSII in the dark-adapted state; F_v'/F_m' – maximal quantum yield of PSII in the light-adapted state; Φ_{PSII} – effective quantum yield of PSII.

Parameters	*R. irregularis*	*G. versiforme*	Control
F_m	0.473 ± 0.050^{a}	0.459 ± 0.112^{a}	0.505 ± 0.032^{a}
F_0	0.126 ± 0.011^{b}	0.127 ± 0.013^{b}	0.138 ± 0.018^{a}
F_m'	0.281 ± 0.043^{ab}	0.252 ± 0.038^{b}	0.312 ± 0.062^{a}
F_0'	0.106 ± 0.011^{b}	0.104 ± 0.011^{b}	0.117 ± 0.019^{a}
F_v/F_m	0.734 ± 0.011^{a}	0.712 ± 0.055^{a}	0.726 ± 0.026^{a}
F_v'/F_m'	0.619 ± 0.039^{a}	0.584 ± 0.025^{b}	0.620 ± 0.048^{a}
Φ_{PSII}	0.397 ± 0.059^{a}	0.387 ± 0.038^{a}	0.332 ± 0.044^{b}
q_P	0.639 ± 0.063^{a}	0.584 ± 0.055^{b}	0.618 ± 0.076^{ab}
NPQ	0.719 ± 0.314^{a}	0.846 ± 0.499^{a}	0.675 ± 0.336^{a}

Chl fluorescence: The F_0, F_m', and F_0' in AM seedlings were all lower than those of NM seedlings. The F_m, F_v/F_m, F_v'/F_m', q_P, and NPQ of the AM seedlings and NM seedlings were not significantly different. However, the F_v'/F_m' and q_P of seedlings colonized with *G. versiforme* were lower than that found in seedlings colonized by *R. irregularis*. Compared with the NM control, the Φ_{PSII} was significantly enhanced in black locust seedlings colonized by either *R. irregularis* or *G. versiforme* (Table 3).

Carbon content and calorific value: AMF increased the carbon content of the roots, stems, and leaves of black locust. However, *R. irregularis* and *G. versiforme* performed differently. The carbon content of the roots and stems of seedlings inoculated with *R. irregularis* were significantly higher than those of NM seedlings, whereas *G. versiforme* only enhanced the leaf carbon content (Fig. 1).

AMF clearly enhanced the calorific value of black locust seedlings (Fig. 2). Calorific values of roots, stems, and leaves of AM seedlings inoculated with *G. versiforme* increased by 10%, 9%, and 7%, respectively, compared with those of NM plants. *R. irregularis* also increased significantly the calorific value of roots, stems, and leaves. However, the calorific value of the stems was lower in the AM seedlings with *R. irregularis* than that of AM seedlings with *G. versiforme*. In addition, a positive correlation was found between the calorific value and the carbon content of black locust ($r = 0.924$, $P<0.05$). The leaves had the highest calorific value and carbon content, followed by the stems and the roots.

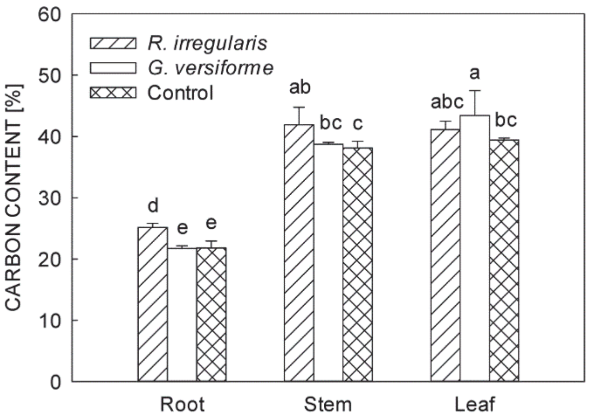

Fig. 1. Effects of *Rhizophagus irregularis* and *Glomus versiforme* on the carbon content of the roots, stems, and leaves of black locust seedlings. Means ± SD labeled with *different letters* are significantly different ($P<0.05$) using *Duncan*'s test; $n = 5$.

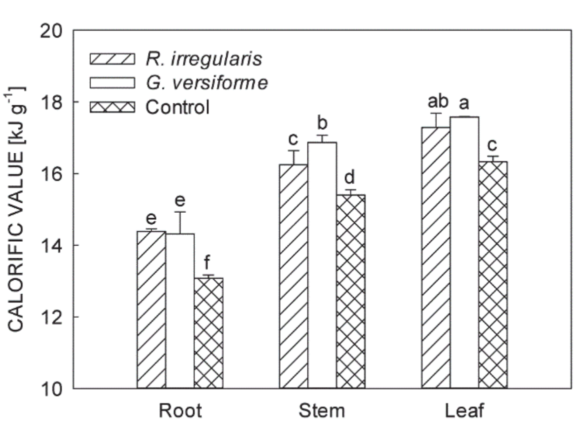

Fig. 2. Effects of *Rhizophagus irregularis* and *Glomus versiforme* on the calorific value of the roots, stems, and leaves of black locust seedlings. Means ± SD labeled with *different letters* are significantly different ($P<0.05$) using *Duncan*'s test; $n = 5$.

Discussion

Numerous papers have reported that mycorrhizal symbiosis can increase the growth and production of host plants (Requena *et al.* 1997, Olesniewicz and Thomas 1999). In our study, AMF mycorrhization of black locust enhanced significantly the growth, biomass yield, photosynthetic ability, and carbon content of the seedlings. Importantly, our study demonstrated that AMF could raise the calorific value of this energy tree.

The AM plants grew faster than NM plants because of their larger root systems and enhanced nutrient uptake facilitated by the mycorrhizal hyphae (Olesniewicz and Thomas 1999, Gong *et al.* 2013). In our study, AM plants had a significantly greater biomass than NM seedlings, and a significantly greater leaf biomass and LA, which is beneficial for carbon assimilation and solar energy absorption.

AMF enhanced Chl content in black locust. It was likely because the AMF increased P and Mg uptake. Chl content is a key factor for plant photosynthesis and closely reflects the photosynthetic ability of plants (Takai *et al.* 2010, Zai *et al.* 2012). In particular, AMF need photosynthetically fixed carbon from the host to maintain their growth. The carbon sink strength due to the AMF symbiosis stimulates the host plants, increasing the photosynthetic rate (Kaschuk *et al.* 2009). The results confirmed that AM seedlings had significantly higher Chl content and P_N than NM seedlings. The greater LA, Chl content, and P_N indicated that carbon assimilation was greater in AM plants than in the NM seedlings (Wright *et al.* 1998, Wu and Xia 2006). The seedlings inoculated with *G. versiforme* had lower C_i and E, which might result from lower g_s and higher CE (Sheng *et al.* 2008). Generally, black locust plants have a lower water requirement than other crops (González-García *et al.* 2011). The lower g_s and E, which might be partly because of closed stomata, suggested that the AM seedlings loose less water.

Chl fluorescence is another widely used and powerful method to study plant photosynthesis (Sheng *et al.* 2008). The parameters of Chl fluorescence can reflect sensitively and accurately photosynthetic ability and energy conversion efficiency (Demmig-Adams *et al.* 2012). In this study, AM plants had lower F_0, F_m', and F_0'. These parameters are relevant mainly to the status of the PSII reaction centers, electron transport, and light-induced activation of enzymes that participate in the photochemical process (Maxwell and Johnson 2000). It is possible that AMF improved the electron transport or enzyme activities of the host plants and, hence, AM seedlings had higher Φ_{PSII} than the controls. It manifested that the AM plants had a higher photochemical efficiency for CO_2 and solar energy fixation. However, other Chl fluorescence parameters, such as F_v/F_m, F_m, F_v'/F_m', q_P, and NPQ were not significantly different in the AM seedlings compared with those in NM seedlings. These parameters are sensitive to stress (Sheng *et al.* 2008); however, the black locust seedlings were not subjected to adverse conditions in our study.

The AM seedlings had a higher carbon content than NM seedlings, which might result from the photosynthetic ability enhanced by AMF (Jongen *et al.* 1996). We found that AMF significantly enhanced the carbon content of the roots, stems, and leaves. It could potentially impact on carbon sequestration even though the carbon content (38.7–41.9%) of the AM stems was lower in this study than that found in other hardwood species (46.2–49.9%) (Lamlom and Savidge 2003). The carbon content of a plant is usually constant, but it can be influenced by various factors, such as plant species, age, and ecological conditions. Generally, mature trees have a higher carbon content than younger trees (Prakash and Murray 1972, Kumar *et al.* 2011). It seems likely that the carbon content of mature AM black locust trees could be higher than that recorded for the 5-month-old seedlings used in this greenhouse experiment.

The AM seedlings absorbed more solar energy and assimilated more CO_2 than NM seedlings. It affects organic matter and carbon content, and greatly contributes to the calorific value of black locust seedlings, because calorific value is influenced by chemical elements and by the composition of the biomass (Demirbas 2005, Fang *et al.* 2013). In our study, the calorific values correlated positively with the carbon content ($r = 0.924$, $P<0.05$). Carbon is the important component of organic and combustible matter, therefore, higher carbon content produces a higher calorific value (Gao *et al.* 2011).

In summary, both AMF species used in this study greatly improved the growth, photosynthesis, carbon content, and calorific value of black locust. The AM seedlings had a higher energy content because of the higher calorific value and greater biomass yield. Moreover, *G. versiforme* enhanced growth and gas exchange of black locust seedlings more effectively than *R. irregularis*.

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