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# *Glomus mosseae* Promotes *Xanthium italicum* Invasion

(*Glomus mosseae* Menggalakkan Pencerobohan *Xanthium italicum*)

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ABSTRACT

*The relationship between mycorrhizal fungi and the invasiveness of alien plants is a controversial issue in plant invasion ecology. In this study, we investigated the influence of the mycorrhizal fungus* Glomus mosseae *on the physiological ecology, growth, and reproductive capacity of* Xanthium italicum *Moretti. We found that the relative chlorophyll content and net photosynthetic rate of the leaves of potted* X. italicum *whose rhizosphere soil was supplemented with* G. mosseae *was higher than those of the control group. Plants in the treatment group had longer root length and plant height; increased root, stem, and fruit biomass; and more male and female inflorescences and fruits relative to control plants. Additionally, the ratio of female to male inflorescence number was close to 2:1 in the treatment group as compared to 1:1 in the control group, suggesting an enhanced reproductive capacity in the former.* G. mosseae *applied to the soil also increased phosphorus absorption and accumulation in the roots, stems, and leaves of* X. italicum*. Thus,* G. mosseae *promotes the growth and reproduction of* X. italicum *and thereby enhances its competitiveness and capacity for colonizing a new habitat.*

*Keywords: Alien plant; biological invasion; Italian cocklebur; mycorrhizal fungi*

ABSTRAK

*Hubungan antara kulat mikoriza dan keserbuan tumbuhan asing adalah isu kontroversi dalam ekologi pencerobohan tumbuhan. Dalam kajian ini, kami mengkaji pengaruh kulat mikoriza* Glomus mosseae *pada ekologi fisiologi, pertumbuhan dan keupayaan pembiakan* Xanthium italicum *Moretti. Kami mendapati bahawa kandungan klorofil relatif dan kadar fotosintesis bersih daun* X. italicum *pot yang tanah rizosferanya dilengkapi dengan* G. mosseae *lebih tinggi daripada kumpulan kawalan. Tumbuhan dalam kumpulan rawatan mempunyai panjang akar dan ketinggian tanaman yang lebih panjang; peningkatan biojisim akar, batang dan buah; dan lebih banyak perbungaan dan buah jantan dan betina berbanding tanaman kawalan. Selain itu, nisbah bilangan perbungaan betina dan jantan mendekati 2:1 dalam kumpulan rawatan berbanding 1:1 pada kumpulan kawalan, menunjukkan peningkatan keupayaan pembiakan pada kumpulan rawatan.* G. mosseae *yang digunakan pada tanah juga meningkatkan penyerapan dan pengumpulan fosforus pada akar, batang dan daun* X. italicum*. Oleh itu,* G. mosseae *mendorong pertumbuhan dan pembiakan* X. italicum *dan dengan itu meningkatkan daya saing dan keupayaannya untuk menjajah habitat baru.*

*Kata kunci: Kerang Itali; kulat mikoriza; pencerobohan biologi; tumbuhan asing*

## INTRODUCTION

Mycorrhiza can improve the ability of alien plants to absorb water and inorganic salts (Brundrett 2009) and enhance their resistance (Wang et al. 2001) and competitiveness (Fumanal et al. 2006) while promoting their growth and reproduction (Chmura et al. 2012), allowing them to successfully invade a new habitat. However, other studies have found no correlation between mycorrhizal fungi and alien plant growth (Bunn 2015). Arbuscular mycorrhizal fungi are widely distributed and affect about 90% of vascular plants on Earth (Harley 2008). Their main functions are to promote the absorption of mineral elements by host plants, increase the resistance of host roots to harmful pathogens, and enhance plant resistance to extreme conditions such as high temperature, high salt, and water shortage. It can also improve photosynthetic efficiency and promote growth and biomass accumulation in the host (Zhi 2003). As one of the most common arbuscular mycorrhizal fungi, *Glomus mosseae* plays an important role in the successful invasion of alien plants.

*Xanthium italicum* Moretti is an annual herbaceous plant of the genus *Xanthium* in the Compositae family exhibiting high reproductive capacity (Lin et al. 2018), strong ecological adaptability (He & Ma 2018) and allelopathic inhibitory activity (Shao et al. 2012), and interspecific competitiveness (Takakura 2010) that has caused serious harm to local agricultural production (Kazinczi 2009). Moreover, the toxicity of stems and leaves (Yang et al. 2006) and the strong adhesion of fruits to animal fur (Qu et al. 2015) threaten the health and development of local livestock. *X. italicum* is native to North America but has spread to many countries and regions in South America, Europe, Asia, and Oceania (Liu et al. 2008), and has been classified as a malignant invasive plant by many countries. It is unclear whether *G. mosseae* has contributed to the expansion of *X. italicum* populations since the mechanism of invasion of this plant is not well understood.

To answer this question, in this study we investigated the influence of *G. mosseae* on the relative chlorophyll content, photosynthetic response, root length, plant height, biomass, number of male and female inflorescences, and phosphorus content of potted *X. italicum*.

## Materials and Methods

### SEED COLLECTION

*X. italicum* exhibits interspecific hybridization and pollination (Lin et al. 2018). In order to eliminate the influence of genetic differences among individuals on the results, a total of 660 mature fruits were collected on the individual self-fertilized and stored them at -20 °C.

### SEEDLING CULTURES

Five plastic pots (80 × 60 × 20 cm) were filled up to 15 cm with river sand sterilized by high pressure. Seeds of healthy *X. italicum* of similar size were evenly sown in the sand with a spacing of 10 cm at a depth of 1 cm, and a total of 175 seeds were sown. The relative humidity of the sand was about 70%. The pots were covered with a colorless transparent plastic film to increase the temperature of the sand and reduce water evaporation, and the pots were placed in an open area of Shihezi University. The plastic film was removed when about 80% of seeds had germinated (i.e. the seedlings were unearthed). The pots were weighed daily to replenish the water (Aminah et al. 2013).

### SEEDLING TRANSPLANTATION

Forty seedlings of the same height were selected for transplantation to plastic pots with a diameter of 25 cm and a height of 28 cm containing sterilized sand (river sand: clay = 1:1), with one seedling per pot. *G. mosseae* spores were incubated up to 5 days at 30°C on PDA (Potato dextrose agar) medium for inoculation. A 5-mm diameter PDA plug of *G. mosseae* was placed on seedling roots in twenty replicate pots. Control seedlings were inoculated with sterile PDA plugs. The Genbank accession number of *G. mosseae* is JF276414. The pots were randomly placed in an open area of the campus with adjacent pots separated by 40 cm to ensure that each seedling received full sunlight. The position of the pots was changed randomly once a week. Each plant was watered daily (200 mL) at 9 am and 8 pm. Measurements were made 60 days after transplantation (Al-Hammadi et al. 2018).

### DETERMINATION OF REATIVE CHLOROPHYLL CONTENT AND GENERATION OF PHOTOSYNTHESIS-PHOTORESPONSE CURVE

The first fully expanded leaf at the top of each plant was selected for the measurement of relative chlorophyll content using a portable chlorophyll meter (SPAD-502 Plus; Konica Minolta, Tokyo, Japan). The photosynthesis-photoresponse curve of the leaves without disease and insect pests were measured using a portable photosynthesis meter (LI-6400; LI-COR, Lincoln, NE, USA). The photosynthetic active radiation gradient was set to 2000, 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 100, 50, 25 and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. The concentration of $CO_2$ was provided by the small cylinder, the concentration was set to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the relative humidity was 70%. Photosynthetic indexes such as light saturation point (LSP) and light compensation point (LCP) were fitted and calculated according to the right-angle hyperbolic modified model of leaves. Ten leaves were sampled for relative chlorophyll content and net photosynthetic rate measurements in the treatment group and control group respectively, and each measurement was repeated three times. (Zhang et al. 2014).

### DETERMINATION OF PLANT GROWTH-RELATED PARAMETERS

Plants were carefully harvested so as not to disturb the roots and washed with distilled water to remove residual soil particles. All growth parameters were recorded first before the plants were dried in the oven for dry weight measurement. Root length and plant height were measured by a ruler, and male and female inflorescences were identified by the naked eye. The biomass (dry weight) of each organ was measured by analytical balance after all organs were dried in a constant temperature oven (70 °C) for 48 h. Both the treatment group and the control group contained ten replicates (He et al. 2017).

DETERMINATION OF PHOSPHORUS CONTENT IN VARIOUS ORGANS OF PLANTS

Dried roots, stems, and leaves were crushed into powder, respectively, and different organs were placed separately. Accurately weighed 0.2 g of dry samples of each organ, put them in a 150 mL flask and added 15 mL mixed acid (concentrated $HNO_3$: $HClO_4$ = 3:1), then, heated and digested them on an electric hot plate in the ventilation cupboard. When the sample blackens and white smoke comes out, removed the triangle bottle and added concentrated $HNO_3$ 1 mL to continue heating and decomposition until the solution becomes clear. Moved the contents into a 100 mL volumetric bottle without damage, and diluted it to the scale with distilled water and shake well to clarify. Absorbed the supernatant 2 mL in a 50 mL volumetric flask, and added distilled water to 30 mL. Added two drops of dinitrophenol indicator, and used 6 mol/L sodium hydroxide solutions to a yellowish color (pH≈7), then added 5 mL ammonium molybdate-ammonium metavanadate chromogenic solution, diluted to the scale with distilled water and mixed well. After 30 min, the absorbance was determined by colorimetry at the wavelength of 440 nm using a spectrophotometer (UV-1240; Shimadzu, Tokyo, Japan), and the content of phosphorus was detected from the standard curve according to the absorbance value. The average value of the ten measurements was calculated (Zhang 2011).

STATISTICAL ANALYSIS

Data were analyzed using SPSS v.20.0 software (SPSS Inc., Chicago, IL, USA). The significance of differences between the treatment and control groups was evaluated by one-way analysis of variance. $P < 0.05$ was considered significant (Li et al. 2019).

## RESULTS AND DISCUSSION

EFFECTS OF *G. mosseae* ON RELATIVE CHLOROPHYLL CONTENT AND NET PHOTOSYNTHETIC RATE OF *X. italicum*

Mycorrhizal fungi can accelerate the synthesis and accumulation of carbohydrates and thus improve the photosynthetic capacity of host plant leaves (Doidy et al. 2012). We found here that the relative chlorophyll content of the leaves of *X. italicum* has grown in soil supplemented with *G. mosseae* was 1.69 times higher than that of control plants ($P < 0.001$; Figure 1).

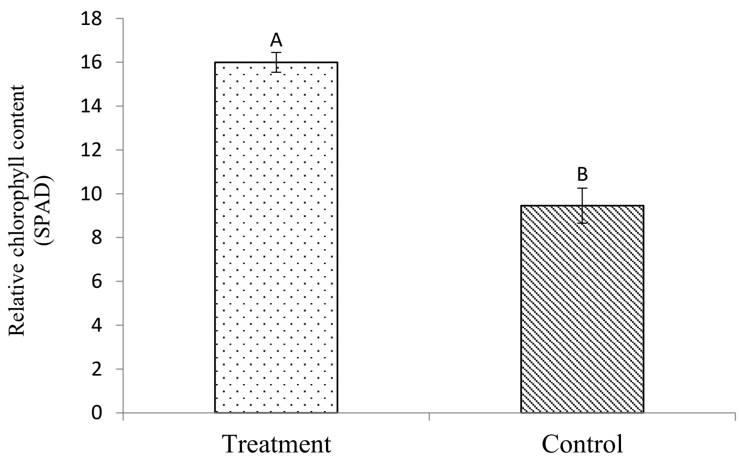


FIGURE 1. Relative chlorophyll content of *X. italicum* has grown in soil with or without *G. mosseae* supplementation. Differences were evaluated by one-way analysis of variance; values with different letters are significantly different ($P < 0.001$) according to Fisher's least significant difference and Duncan tests (n = 10)

In addition, individuals in the treatment group reached the light compensation point at a light intensity of about 20 lx as compared to 100 lx for the control group, indicating that the photosynthetic capacity of *X. italicum* under weak light was enhanced by the presence of *G. mosseae*. The net photosynthetic rate of the leaves of *G. mosseae*-treated

*X. italicum* was higher than that in control plants under strong or weak light (Figure 2). Thus, the photosynthetic capacity of *X. italicum* leaves was enhanced by adding *G. mosseae* to the soil. This is consistent with earlier reports (He et al. 2007; Zai et al. 2012) and may be explained by the fact that the accumulation of organic matter in the plant was increased by symbiotic interaction with the fungus (Subramanian et al. 2010). This process may help to increase the relative chlorophyll content in the leaves of *X. italicum* in order to capture more light energy and enhance its photosynthetic ability.

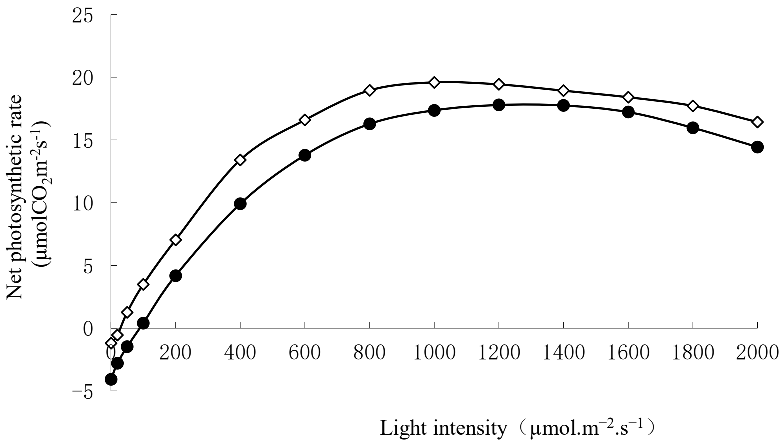


FIGURE 2. Net photosynthetic rate of *X. italicum* has grown in soil with or without *G. mosseae*. Open squares and closed circles represent treatment and control groups, respectively

EFFECT OF *G. mosseae* ON *X. italicum* GROWTH

Arbuscular mycorrhizal fungi can enhance the capacity of plant roots to absorb soil moisture and nutrients, thereby, improving water metabolism and promoting plant growth through rhizobial mycelia (Liu et al. 2009). *G. mosseae* was shown to increase dry matter accumulation in cucumber, tomato, and tobacco seedlings (Liu et al. 2014; Sun et al. 2016; Wang 2003). We found that root length and plant height were increased in the treatment group relative to the control group ($P = 0.003$ and $P < 0.001$, respectively). Similarly, root and stem were 25.6 and 41.8% longer, respectively, in treated as compared to control plants (Figure 3).

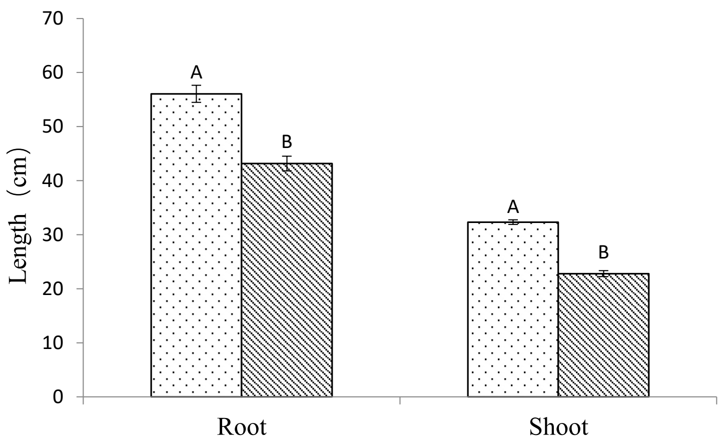


FIGURE 3. Root length and plant height of *X. italicum* has grown in soil with or without *G. mosseae*. Points and lines with bars represent treatment and control groups, respectively. Differences were evaluated by one-way analysis of variance; values with different letters are significantly different ($P < 0.01$) according to Fisher's least significant difference and Duncan tests ($n = 10$)

*G. mosseae* also increased the biomass of the roots, stems, and fruits (P = 0.004, P = 0.006, and P < 0.001, respectively), with 1.6 times higher fruit biomass in the treatment as compared to the control group. On the other hand, there was no significant difference in leaf biomass between the two groups (P = 0.356; Figure 4). The average number of female and male inflorescences per plant was 5.9 and 2.75 times greater in the treatment group than in control plants (P < 0.001), with a female to male inflorescence ratio of 2:1 in the former and of 1:1 in the latter (Figure 5). Thus, the association of *G. mosseae* with *X. italicum* not only promoted growth and biomass accumulation, but also increased the number of female and male inflorescences and fruiting capacity. It is especially noteworthy that *G. mosseae* potently stimulated the differentiation of female inflorescences and the reproductive capacity of the host plant.

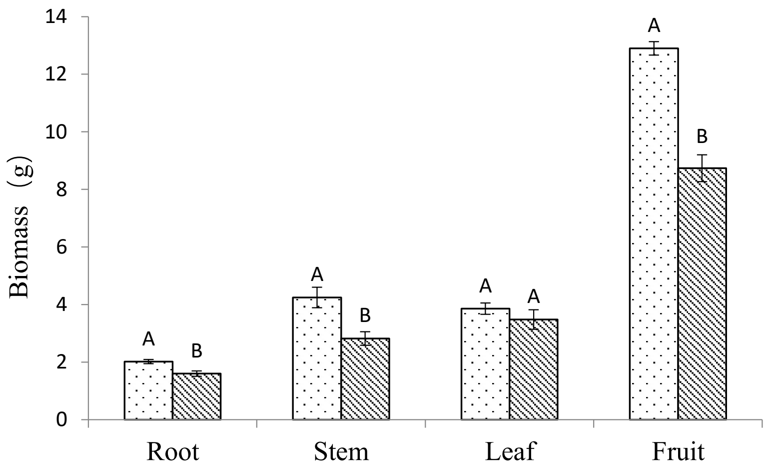


FIGURE 4. Dry weight of organs of *X. italicum* has grown in soil with or without *G. mosseae*. Points and lines with bars represent treatment and control groups, respectively. Differences were evaluated by one-way analysis of variance; values with different letters are significantly different (P < 0.01) according to Fisher's least significant difference and Duncan tests (n = 10)

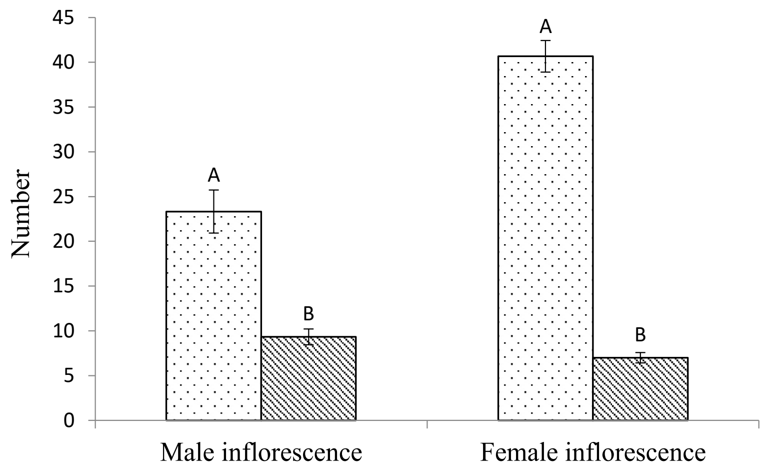


FIGURE 5. The male and female inflorescence number of *X. italicum* has grown in soil with or without *G. mosseae*. Points and lines with bars represent treatment and control groups, respectively. Differences were evaluated by one-way analysis of variance; values with different letters are significantly different (P < 0.001) according to Fisher's least significant difference and Duncan tests (n = 10)

EFFECT OF *G. mosseae* ON PHOSPHORUS CONTENT IN VARIOUS ORGANS OF *X. italicum*

Phosphorus plays an important role in plant respiration and carbohydrate and fat metabolism. Arbuscular mycorrhizal fungi have been shown to enhance the ability of host plants to assimilate low mobility elements such as phosphorus, zinc, and copper in the soil (Marschner 1994), and facilitate the absorption of phosphorus specifically. We determined that phosphorus accumulation in the roots, stems, and leaves were increased in plants grown in *G. mosseae*-supplemented soil relative to control plants (P = 0.011, P < 0.001, and P = 0.08, respectively; Figure 6), as previously reported (Lee et al. 2014). This may be explained by the activation of insoluble phosphate in the soil as a result of a pH change caused by acid substances such as citric acid secreted by *G. mosseae*, which improved phosphorus uptake and utilization by *X. italicum* (Sun et al. 2016). Nivelle et al. (2018) also reported that some mycorrhizal fungi have the ability to dissolve phosphorus in soil, which is also a fundamental reason why some invasive plants can be used as pioneer plants in special habitats.

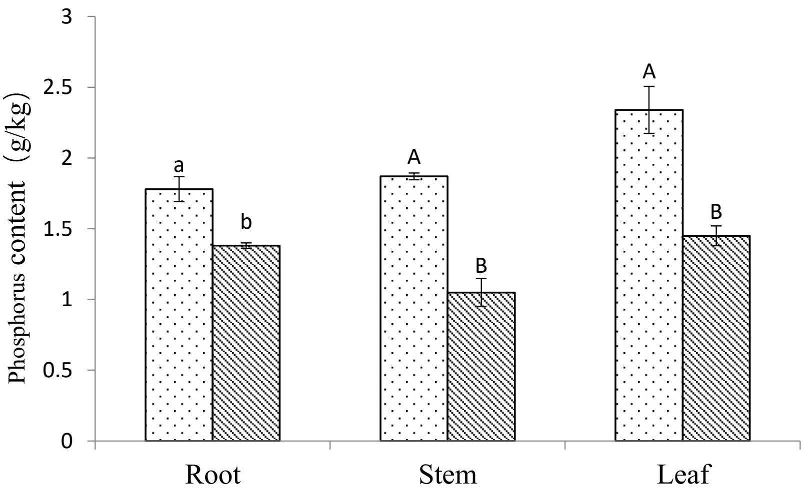


FIGURE 6. Phosphorus content of *X. italicum* has grown in soil with or without *G. mosseae*. Points and lines with bars represent treatment and control groups, respectively. Differences were evaluated by one-way analysis of variance; values with different letters are significantly different (P < 0.05) according to Fisher's least significant difference and Duncan tests (n = 10)

Once alien plants successfully settle in the invading area, it is possible to form dominant species through rapid population expansion, which not only results in the change of habitat plant community structure and the loss of biodiversity, but also has a far-reaching and complex impact on the physical and chemical properties of soil and the microbial community and function. Successful invasion by plants depends on the biological characteristics of the species and the invisibility of the habitat - i.e., the abiotic environment and resistance to biological invasion (Chaudhary 2015; Li 2001). In addition to a lack of natural competitors (Cronin et al. 2016) and an empty niche (Johnson 2016) in new areas, mycorrhizal fungi are one of the main determinants of the successful invasion, survival, and expansion of alien plants (Peng 1999).

## CONCLUSION

The results of this study demonstrate that the arbuscular mycorrhizal fungus *G. mosseae* contributes to the establishment of the alien plant species *X. italicum* in new areas by increasing relative chlorophyll content, net photosynthetic rate, and phosphorus accumulation in roots, stems, and leaves of the plant, thereby promoting its growth and survival and conferring it with a competitive advantage over native plants. Moreover, *G. mosseae* increased the number of male and female inflorescences and the ratio of female to male inflorescences, indicating an enhanced reproductive capacity in *X. italicum*. These results provide insights into the relationship between a mycorrhizal fungus and its host plant that can be useful for developing measures to limit the colonization of new areas by alien plant species that can disrupt the local ecological balance.

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