Pol. J. Environ. Stud. Vol. 31, No. 5 (2022), 4399-4411
DOI: *10.15244/pjoes/148184* ONLINE PUBLICATION DATE: 2022-07-04

Original Research

The Invasive Weed *Xanthium italicum* Significantly Decreases *Glycyrrhiza uralensis* Yield and Severely Reduces Its Medicinal Quality

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Received: 28 November 2021
Accepted: 9 April 2022

Abstract

Italian cocklebur (*Xanthium italicum* Moretti) invades liquorice farmlands, but specific consequences of the invasion remain unclear. Therefore, in this study, we simulated soil fertility levels of liquorice farmlands and performed replacement experiments to compare the relative competition intensity between *X. italicum* and Chinese liquorice (*Glycyrrhiza uralensis*), and investigated the differences in the effects of planting patterns (monoculture and mixture) on the growth and reproduction of the two species. Under monoculture treatment, the growth of *X. italicum* was more than that of *G. uralensis*. Vegetative and reproductive growth of *X. italicum* was significantly increased under mixture culture treatment compared to that under monoculture treatment; however, growth and clonal reproduction ability of *G. uralensis* were significantly suppressed. The biomass and contents of secondary metabolites of *G. uralensis* were significantly decreased under mixed culture treatment, and rhizome development was completely inhibited after mixed planting with *X. italicum*. The differences in vegetative growth between the two plants became larger under mixed culture treatment. Therefore, *X. italicum* exhibited significantly higher competitiveness than *G. uralensis* when coexisting within a community, and the *X. italicum* exerted significantly negative effects on the growth, reproduction, nodule development, yield of medicinal materials and contents of medicinal secondary metabolites of *G. uralensis*.

Keywords: Italian cocklebur, *Glycyrrhiza uralensis*, invasive weed, mixture culture, competitiveness

Introduction

Biological invasion is an international environmental problem [1]. With the acceleration of globalization, alien species have seriously threatened the integrity of the invaded ecosystems [2]. Alien plants are an important group of invasive species, exhibiting strong competitive and reproductive abilities; these characteristics enable them to quickly plunder the resources needed for survival by the neighbouring plants and to quickly become dominant species in communities. This phenomenon usually impacts the growth and development of the native plants, and may even lead to their extinction in local populations,

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resulting in the reduction of biodiversity and serious damage to the ecological balance of the local environment [3]. The plant community structure of farmland habitats is simple, with their stability being low and their resistance to invasive plants being weak, which makes farmland ecological environments vulnerable to the invasion by foreign plants. As a result, invasive plants often have strong negative impact on the growth, reproduction, and yield of many crops [4, 5].

Xanthium italicum Moretti, an annual herb of the family Compositae, is native to North America, but has become a global invasive weed and is now widely distributed in the middle latitude areas of both the Eastern and Western hemispheres [6]. It was first discovered in China in the 1990s and has become widely distributed in many provinces, including Beijing, Liaoning, Hebei, Shandong, Shanxi, and Xinjiang, with a tendency to spread throughout the country [7, 8]. *X. italicum* generates a very large seed set, exhibits strong characteristics of ecological adaptation, and has impressive resource competition abilities. This allows it to often form a high-density, single-species dominant population in communities and seriously impact the growth of neighbouring plants, which threatens local biodiversity and causes serious damage to local agricultural production and husbandry development [9-11]. Previous studies have reported effects of interspecific competition between *X. italicum* and local crops, including corn, cotton, soybean, and oil sunflower. However, all these studies focused on the accumulation of crop biomass and economic organ production [12-14]. As a result, the impact on the content of plant secondary metabolites, especially the accumulation of active ingredients in medicinal plants, remains unknown.

Glycyrrhiza uralensis Fisch. is a Chinese traditional herb from the Leguminosae family [15]. The roots and rhizomes of *G. uralensis* contain many secondary metabolites that have a variety of properties [16], including antimicrobial, antioxidant, and cancer cell proliferation inhibition [17]. Meanwhile, the stems and leaves of *G. uralensis* are also excellent forage for sheep and cattle, and are widely used in livestock husbandry [18]. While the above-ground plant parts can be used as forage, the underground parts can be used for medicinal purposes.

The demand for liquorice in the international market has been increasing in recent years, but the dwindling availability of wild *G. uralensis* cannot meet this demand. Therefore, large-scale cultivation of liquorice has been carried out in China. Cultivated liquorice is becoming the main source of income for many local farmers. However, the Italian cocklebur (*X. italicum*) has invaded liquorice farmlands, leading to the co-existence of the two species. We observed that liquorice plants growing near Italian cocklebur plants are generally short in size; however, whether this phenomenon is due to competition for resources with the Italian cocklebur is uncertain.

As a cultivated medicinal plant, close attention should not only be paid to the output of the economically beneficial parts of *G. uralensis*, but also on the content of its medicinal ingredients. Whether invasion of *X. italicum* affects the stem and leaf growth of *G. uralensis* or the yield and quality of medicinal materials (roots and rhizomes) should be investigated. It is currently unclear whether farmland interspecific competitions of *X. italicum* and *G. uralensis* are affected by soil fertility levels. Therefore, in the current study, we performed replacement series experiments to explore the impact of interspecific competitiveness of *X. italicum* on the yield and quality of *G. uralensis* under different soil fertility conditions. Under controlled conditions, pot experiments simulating different soil fertilizer conditions of liquorice farmlands were performed. The results of this study would provide experimental evidence for evaluating the consequences of Italian cocklebur invasion on farmland liquorice populations.

Material and Methods

Site of the Experiment

Pot experiments were conducted on the campus of Shihezi University (44°31′10″N, 86°06′94″E) in Xinjiang Province, China at an altitude of 448 m above sea level, where the mean annual temperature was 7.8ºC; mean annual precipitation was 188 mm; and the average annual evaporation was 1820 mm.

Plant Material

Fully ripened *X. italicum* fruits (infructescence with bracts) were collected from the suburbs of Shihezi, Xinjiang Province, China (44°27′31″N, 85°99′81″E) in October 2019. Fully ripened *G. uralensis* seeds were collected from Yuli, Xinjiang (41°32′79″N, 86°30′44″E) in September 2019. The collected seeds were naturally dried in the laboratory and stored at 4°C. The plants were identified by Professor Miao Ma of the College of Life and Science, Shihezi University, Shihezi, China, and voucher specimens of *X. italicum* (herbarium number: 20191045) and *G. uralensis* (herbarium number: 20191071) were deposited at the herbarium of Shihezi University (SHI), China.

Methods

Seed Pre-treatment

To ensure synchronization of seed germination, the seeds were pre-treated prior to use. *X. italicum* seeds of the same size were selected and soaked in distilled water for 12 h at 25ºC before sowing. *G. uralensis* seeds of the same size were soaked in 98% sulfuric acid solution for 30 min, rinsed with running water, and then soaked in distilled water for 6 h at room temperature.

Experimental Design

On April 15, 2019, seeds of the two plants were placed under 12 h of illumination (light intensity, 200 μmol·m^{-2}·s^{-1}) at 25ºC and 12 h of darkness at 20ºC in a light incubator (GXZ-430A, Ningbo Jiangnan Instrument Factory, Ningbo, China) for seed germination. Liquorice farmland soil (sandy soil) was used as the cultivation substrate. The soil properties were as follows: total nitrogen content, 0.268 g·kg^{-1}; total phosphorus content, 0.0855 g·kg^{-1}; total potassium content, 5.72 g·kg^{-1}; available nitrogen content, 43.59 mg·kg^{-1}; available phosphorus content, 4.1 mg·kg^{-1}; quick-acting potassium content, 119.09 mg·kg^{-1}; organic matter content, 5.81 g·kg^{-1}; and pH, 7.5. Seedlings of the same size of each plant were selected after emergence of the 4th true leaf and transplanted into plastic pots (30 cm diameter × 30 cm height). According to conventional cultivation density of farmland liquorice [19], total planting density was four plants per pot. Two fertilization treatments were designed to simulate the fertility level of different farmland soils; low-fertilizer treatment (LF: 1.1 g urea, 1.2 g superphosphate, and 0.9 g potassium sulphate per pot) and high-fertilizer treatment (HF: 2.2 g urea, 2.4 g superphosphate, and 1.8 g potassium sulphate per pot). Replacement experiments were also performed [20], which consisted of two types of cultivation patterns, monoculture treatment and interplanting treatment (Fig. 1). The monoculture treatment included either four *X. italicum* seedlings or four *G. uralensis* seedlings per pot. Each of the four individual plants were positioned at the four vertices of a square. The interplanting treatment included two *X. italicum* seedlings and two *G. uralensis* seedlings that were interplanted at the four vertices of the square. Thus, with LF and HF treatments, and three combinations of the two species (*X. italicum* and *G. uralensis* ratios of 4:0, 2:2, and 0:4), there a total of six treatment groups were analysed in this study (Table 1). Each combination had 15 replicates. During the experiment, the pots were weighed and water was added daily to keep the moisture content of the soil at 70%. The pots were randomly placed in an open area on the campus of Shihezi University. To avoid mutual shading, the distance between adjacent pots was 60 cm. The positions of the pots was randomly changed every other week. All the plants were harvested at the end of the *X. italicum* growing period.

Data Collection

Measurement of Morphological Characteristics

After the plants were harvested, the roots were rinsed with water and the height, crown width, stem diameter of each plant, and rhizome length of the *G. uralensis* plants was measured; the numbers of rhizobia, rhizomes, and buds of *G. uralensis* were also counted. A WinRHIZO LA 2400 root scanner (Canada) was used to measure the total root length, root projection area, total root surface area, and average root

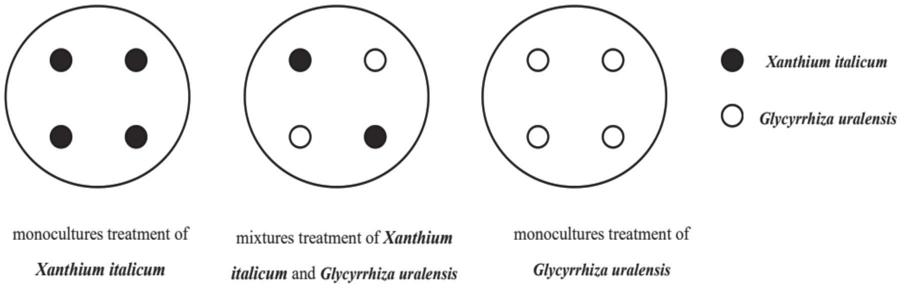

Fig. 1. The planting diagram of *Xanthium italicum* and *Glycyrrhiza uralensis*.

Table 1. Different planting patterns and fertilization treatments combinations.

Planting combination mode	Fertilization treatments	
	LF	HF
Monocultures treatment of *Xanthium italicum*	Monocultures(*Xanthium italicum*) + LF	Monocultures(*Xanthium italicum*) + HF
Monocultures treatment of *Glycyrrhiza uralensis*	Monocultures(*Glycyrrhiza uralensis*) + LF	Monocultures(*Glycyrrhiza uralensis*) + HF
Mixtures trecatment of *Xanthium italicum* and *Glycyrrhiza uralensis*	Mixtures + LF	Mixtures + HF

Note: LF represents low-fertilizer treatment, HF represents high-fertilizer treatment.

diameter of the liquorice plants, and the average root values were calculated.

Measurement of Ecological Traits

The numbers of newly emerged *X. italicum* fruits and male inflorescences were counted every 2 d starting at the beginning of August 2020, as the anthers had begun to disperse pollen. The average number of seeds and male inflorescences per plant was calculated. Six fully unfolded leaves at the top of each plant were randomly selected and the leaf areas were measured using a WinRHIZO LA 2400 root scanner. The leaves were then placed in an oven at 60°C and dried to a constant weight. Mass of the leaves was weighed and the specific leaf area (*SLA*) was calculated using the following formula:

$$\text{SLA} = \text{Leaf Area (cm}^2\text{) / Leaf Dry Weight (g)}$$

The root, stem, leaf, fruit (*X. italicum*), and rhizome (*G. uralensis*) were separated and dried in an oven at 60ºC to a constant weight. The biomass of each part was weighed, and their average values calculated.

Estimation of Secondary Metabolite Concentration

The dried roots and rhizomes of *G. uralensis* were mixed, crushed to a powdered form, and passed through a 40-mesh sieve. The total flavonoid content was determined using an ultraviolet spectrophotometer (UV-1900, Shimadzu, Japan) according to the method reported by Guo et al. (2015) [21]. A standard stock solution of liquiritin was prepared and the absorbance of serially diluted liquiritin was measured at 400 nm; a standard curve was plotted based on the absorbance values, using the following regression equation: $Y = 0.0487435X + 0.167866$, $R^2 = 0.9950$. The results showed that the linear range of flavonoid detection was 0-26.7 $\mu g \cdot mL^{-1}$. Then, 0.100 g of powder of each treatment group sample was weighed and placed into 10 mL test tubes, and 7 mL of 60% ethanol was added to each tube. The tubes were placed into a 300W ultrasonic extractor (KQ-300E, Kunshan Ultrasonic Instrument Co., Ltd., China) and ultrasonically extracted for 2 h. The samples were then centrifuged at 12,000 rpm and 4ºC for 30 min, and the supernatants were collected and filtered through 0.25 µm microfiltration membranes. A 2 mL aliquot of each sample was mixed with 2 mL of 10% potassium hydroxide and incubated at room temperature for 5 min. The samples were diluted to 10 mL with 70% ethanol, and their absorbance was measured at 400 nm. The absorbance of each treatment sample was measured three times, and the average total flavonoid content was calculated.

Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was used to determine glycyrrhizic acid, glycyrrhetinic acid, liquiritin, liquiritigenin, isoliquiritigenin, and glabridin concentrations, using a Water's Acquity UPLC system combined with an MS detector [22, 23]. Three samples were analysed for each treatment, and each sample was measured in triplicate. UPLC-MS/MS separations were performed on a 50 mm × 2.1 mm UPLC BEH C18 column (particle size: 1.7 µm) under the following conditions: column flow rate, 0.3 mL/min; column temperature, 30ºC; and injection volume, 1 µL. The solvents used for this experiment were 0.1% formic acid-water (A) and acetonitrile (B), and gradient elution was performed as per the following conditions: 0-3.0 min, 20-98% B; 3.0-4.5 min, 98% B; 4.5-5.0 min, 98%-20% B; and 5.0-7.0 min, 20% B. An electrospray ionization source (ESI) was used for MS analysis, and the multi-reaction detection mode (MRM) was used for content determination. For MS analysis, the desolvation temperature was 450ºC, source temperature was 150ºC, desolvation gas flow rate was 800 $L \cdot h^{-1}$, cone gas flow rate was 150 $L \cdot h^{-1}$, and capillary voltage was 2300 V. The product ions of the six compounds were optimized based on the collision-induced dissociation of their parent ions. The results of quantitative analysis of ion pairs, fragmentation voltage, and collision energy are shown in Table 2.

Measurement of Competition Intensity

Relative yield (*RY*) and relative competition intensity (*RCI*) were calculated to determine competition intensity according to the following formulas: $RY_i = Y_{ij}/(Y_{ii} \times Z_i)$ [20] and $RCI_i = (Y_{ii} - Y_{ij})/Y_{ii}$ [24], where Y_{ij} is the biomass of species i when grown with species j, Y_{ii} is the mean biomass of species i when grown in monoculture, and Z_i is the proportion of species i in the mixed culture. An RY_i value of 1.0 indicated equal intraspecific and interspecific competition intensities between the two species. An RY_i value >1.0 indicated that intraspecific competition was greater than interspecific competition. Conversely, an RY_i value <1.0 indicated that intraspecific competition was smaller than interspecific competition. *RCI* values range between 0 and 1. The greater the RCI_i value, the lower the yield of species i, the lower the competitiveness of species i in the mixed culture (ij) community, and the greater the influence of species j on species i. An RCI_i value of 1 indicated that species i has been excluded, while an RCI_i value of 0 indicated that species i had no effect on the yield of species j. An RCI_i value <0 indicated that the existence of species j in the mixed culture could promote the growth and the yield of species i.

The relative increase or decrease in the biomass of a particular species growing in the mixed culture, in relation to the expected biomass based on the species proportion, was expressed as "aggressivity" (*A*) [25]. A was calculated for a particular species as follows: $A_i = RY_i - RY_j$. An $A_i > 0$ indicated that the competitiveness of species i was greater than that of species j. An A_i value = 0 indicated that the competitiveness of species i and j were the same; $A_i < 0$ indicated that

Table 2. Optimized mass spectrometry conditions, regression equations and correlation coefficients for 6 compounds.

Secondary metabolite	Parent ion ($m \cdot z^{-1}$)	Daughter ion ($m \cdot z^{-1}$)	Ionization mode	Voltage (V)	Collisional energy (eV)	Retention time (min)	Regression equation	R^2	Linear over ($ng \cdot mL^{-1}$)
Glycyrrhizic acid	821.2	350.9*	–	62	42	2.48	Y = 87.2 X – 3.76	0.9992	1.0-978.0
		113.0		62	56				
Glycyrrhetinic acid	469.2	355.1*	–	100	44	3.81	Y = 136.8X – 122.4	0.9990	1.4-993.2
		409.2		100	46				
Liquiritin	417.0	254.9*	–	52	20	1.00	Y = 750.9 X + 356.9	0.9999	1.0-992.3
		134.9		52	30				
Liquiritigenin	257.2	137.0*	+	92	24	1.97	Y = 509.2X + 925.6	0.9980	0.9-962.5
		147.0		92	18				
Isoliquiritigenin	257.0	136.9*	+	2	24	2.47	Y = 8775.1X + 2617.2	0.9993	1.1-985.6
		147.0		2	18				
Glabridin	323.0	201.0*	–	2	24	3.25	Y = 591.8 X + 223.3	0.9994	1.1-982.7
		134.9		2	18				

Note: "*" represents quantitative ion, R^2 represents correlation coefficient.

the competitiveness of species *i* was less than that of species *j*.

Data Analyses

Data were analysed using SPSS software, version 19.0 (IBM Corp., New York, NY, USA). One-way analysis of variance (ANOVA) was used to analyse the difference between different planting patterns or different fertilization treatments. The t-test was used to compare the difference between *RY* and 1, and between *RCI*, *A*, and 0. Origin 2016 software (OriginLab, Northampton, MA, USA) was used to generate the graphs.

Results

Comparison of Growth between *X. italicum* and *G. uralensis* Under Monoculture Treatment

Under monoculture treatment, a significant difference in growth was observed between *X. italicum* and *G. uralensis* ($P<0.01$). The height, crown width, stem diameter, SLA, root biomass, stem biomass, leaf biomass, and total biomass of *X. italicum* were 3.25-, 0.87-, 4.19-, 0.67-, 0.48-, 19.19-, 4.51-, and 3.64-fold more than those of *G. uralensis*, respectively, under LF treatment (Fig. 2). The height, crown width, stem diameter, SLA, root biomass, stem biomass, leaf biomass, and total biomass of *X. italicum* were 3.44-, 1.15-, 3.49-, 0.75-, 0.46-, 16.61-, 3.17-, and 3.98-fold more than those of *G. uralensis*, respectively, under HF conditions (Fig. 2).

Effects of Planting Pattern on *X. italicum* and *G. uralensis*

Effect of Cultivating Patterns on Vegetative Growth and Reproductive Potential

Cultivating pattern had a significant effect on the growth and development of *X. italicum* and *G. uralensis* ($P<0.05$). Under LF and mixture treatment conditions, the height and crown width of *X. italicum* increased by 20% and 85%, respectively, compared to that under LF and monoculture treatment conditions (Fig. 2). Furthermore, the numbers of *X. italicum* seeds and male inflorescences increased by 44% and 77%, respectively, under mixed cultivation compared to those under monoculture treatment (Fig. 3). Under HF and mixture treatment conditions, the height and crown width of *X. italicum* increased by 22% and 13%, respectively, compared to those under HF and monoculture treatment (Fig. 2). In addition, the numbers of *X. italicum* seeds and male inflorescences increased by 58% and 44%, respectively, under mixed cultivation compared to those under monoculture treatment (Fig. 3). The cultivating pattern also significantly affected the clonal reproductive potential of *G. uralensis* ($P < 0.05$). The average number of *G. uralensis* rhizomes under monoculture treatment was 1, and the average number of rhizome buds was 5. However, the number of *G. uralensis* rhizomes under mixture treatment was 0, and the development of rhizomes was completely inhibited, while the number of rhizome buds under LF and HF treatments was 1 (Fig. 3). Under LF and mixture treatment, the height, crown width, total root length, root projection area, total root surface area, average root diameter, and nodules number of *G. uralensis* were reduced by 46%, 58%, 39%, 62%, 62%, 41%, and 81%, respectively, compared to those under LF and monoculture treatment. Under HF and mixture treatment, the height, crown width, total root length, root projection area, total root surface area, average root diameter, and nodule number of *G. uralensis* were 44%, 60%, 48%, 54%, 67%, 36%, and 95% less, respectively, compared to those under HF and monoculture treatment (Fig. 2, Table 3).

Effect of Cultivating Patterns on Biomass Accumulation

Cultivating pattern significantly affected the biomass accumulation of various parts of *X. italicum* ($P<0.01$). Under LF and mixture treatment, the root biomass, stem biomass, leaf biomass, fruit biomass, and total biomass of *X. italicum* increased by 166%, 166%, 149%, 38%, and 140%, respectively, compared to those under LF and monoculture treatment. Under HF and mixture treatment, the root biomass, stem biomass, leaf biomass, fruit biomass, and total biomass of *X. italicum* increased by 176%, 106%, 175%, 51%, and 108%, respectively, compared to those under HF and monoculture treatment (Fig. 2).

Cultivating pattern also significantly affected the biomass accumulation of *G. uralensis* ($P<0.01$). Under LF and mixture treatment, the root biomass, stem biomass, leaf biomass, and total biomass of *G. uralensis* were reduced by 84%, 86%, 91%, and 85% respectively, compared to those under LF and monoculture treatment. Under HF and mixture treatment, the root biomass, stem biomass, leaf biomass, and total biomass of *G. uralensis* were reduced by 88%, 93%, 89%, and 89%, respectively, compared to those under HF and monoculture treatment (Fig. 2).

Effects of Cultivating Patterns on Production of G. uralensis Secondary Metabolites

Planting patterns significantly affected the accumulation of secondary metabolite in the underground organs of *G. uralensis* ($P<0.01$). Under LF and mixture treatment, the total flavonoid, glycyrrhizic acid, glycyrrhetinic acid, liquiritin, liquiritigenin, isoliquiritigenin, and glabridin content in the root and rhizome of liquorice decreased by 4%, 5%, 62%, 18%, 4%, 41%, and 20%, respectively, compared to that under LF and monoculture treatment (Fig. 4). Under HF and mixture treatment, the contents of the total flavonoid, glycyrrhizic acid, glycyrrhetinic acid, liquiritin, liquiritigenin, isoliquiritigenin, and glabridin were decreased by 29%, 57%, 71%, 62%, 40%, 88%, and 46%, respectively, compared to those under LF and monoculture treatment (Fig. 4).

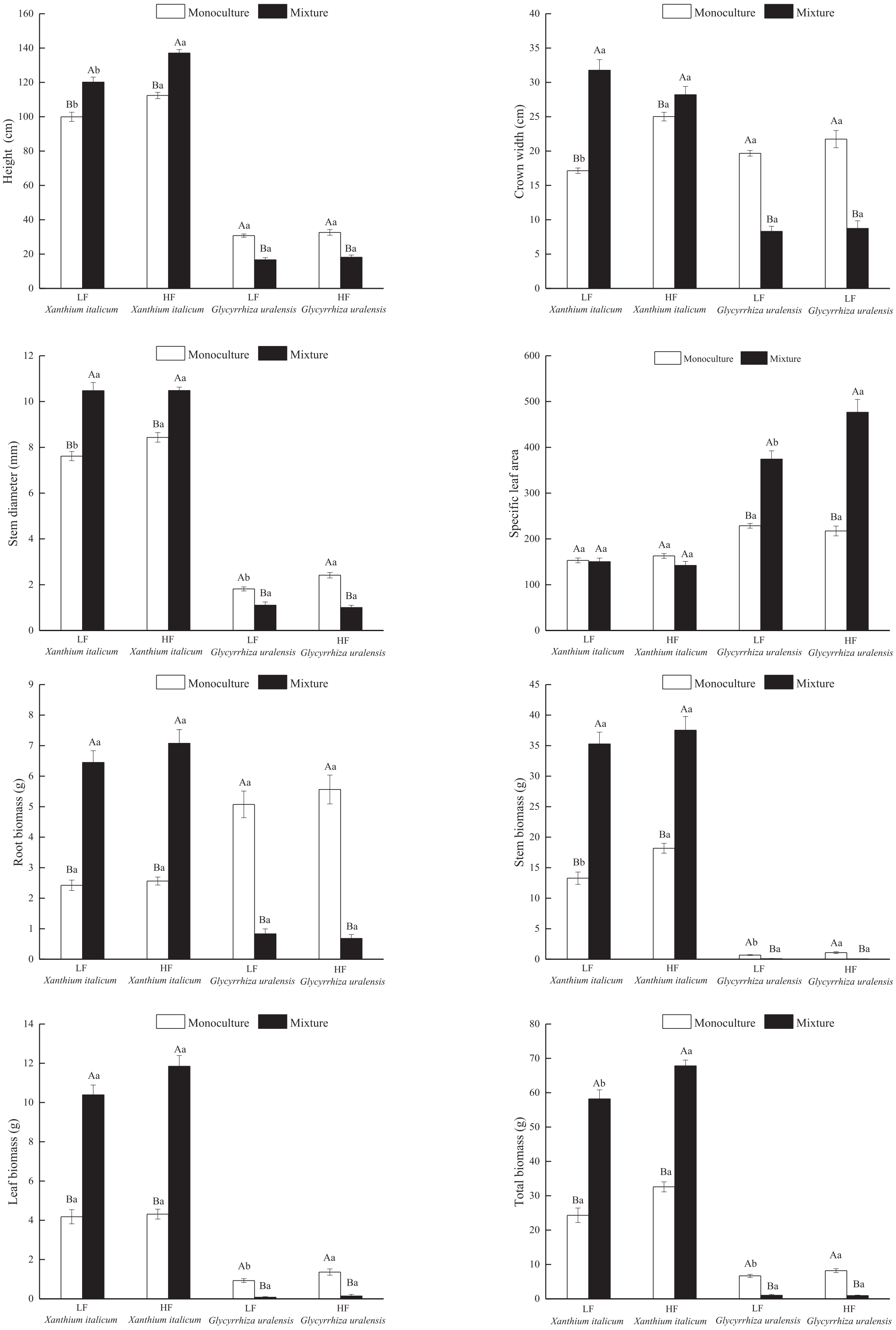

Fig. 2. The growth-related parameters of *Xanthium italicum* and *Glycyrrhiza uralensis* (mean±SE), the different capital letters means significant difference between monoculture and mixture treatments, the different lowercase letters means significant difference between LF (low-fertilizer) and HF (high-fertilizer) treatments.

Table 3. Effects of cultivating patterns on the root growth of *Glycyrrhiza uralensis*.

Traits	LF		HF	
	Monocultures	Mixtures	Monocultures	Mixtures
Total root length/ cm	349.52±16.5 Ab	213.12±10.5 Bb	457.48±22.3 Aa	239.4±10.6 Ba
Root projection area/cm^2	30.16±1.5 Ab	11.5±0.7 Ba	40.86±1.5 Aa	18.95±0.5 Ba
Total root surface area/ cm^2	100.49±2.6 Ab	38.56±1.8 Bb	129.92±6.6 Aa	43.33±3 Ba
Average root diameter/ mm	0.91±0.02 Ab	0.54±0.02 Ba	1.06±0.03 Aa	0.68±0.03 Ba
Number of root nodule	10.75±0.6 Ab	2±0.2 Ba	28.2±1.49 Aa	1.5±0.1 Ba

Note: The different capital letters means significant difference between monoculture and mixture treatments; different lowercase letters means significant difference between LF (low-fertilizer) and HF (high-fertilizer) treatments.

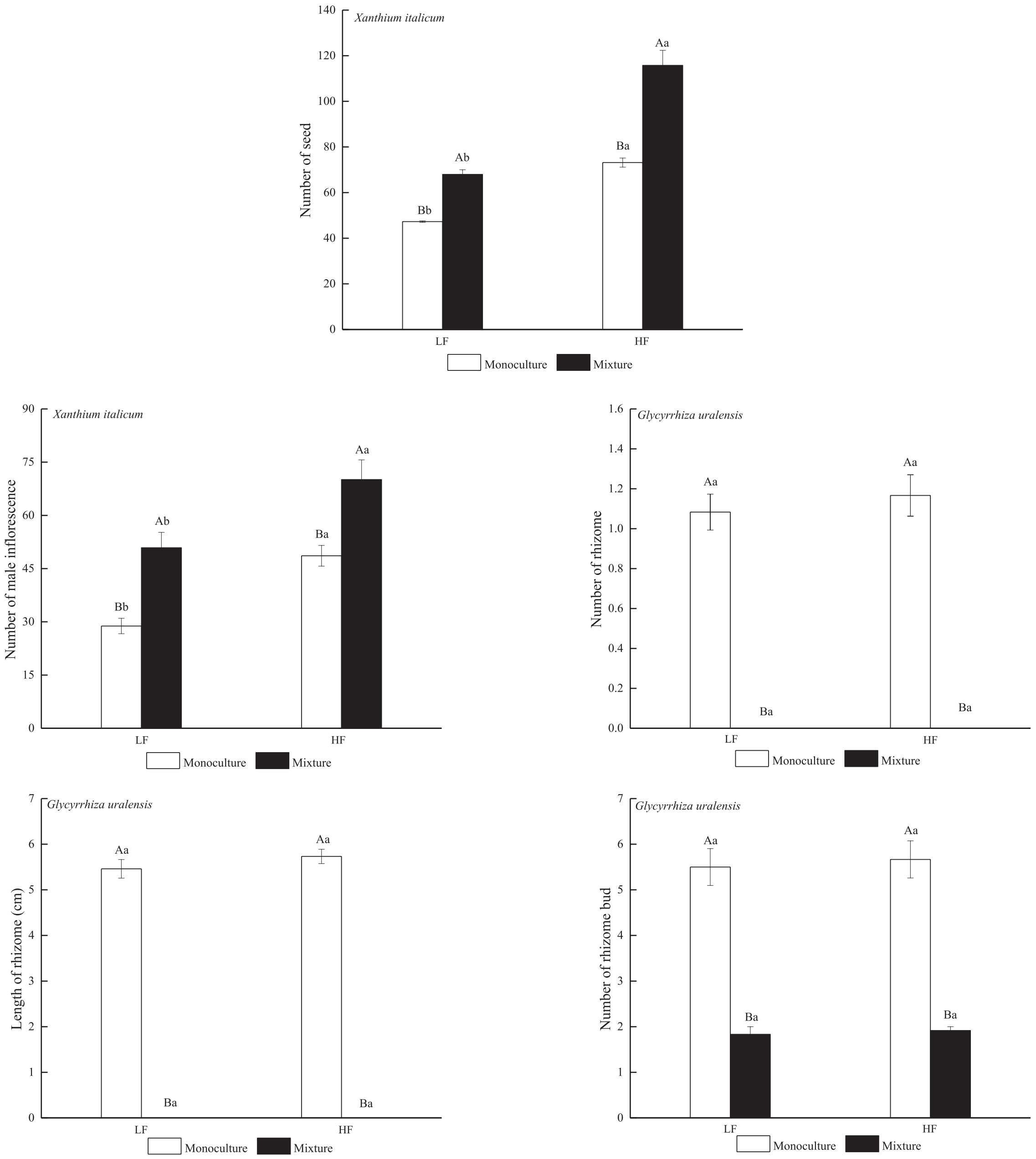

Fig. 3. Effects of cultivating patterns on the reproductive potential of *Xanthium italicum* and *Glycyrrhiza uralensis* (mean±SE), the different capital letters means significant difference between monoculture and mixture treatments, the different lowercase letters means significant difference between LF (low-fertilizer) and HF (high-fertilizer) treatments.

Comparison of Growth of *X. italicum* and *G. uralensis* Under Mixture Treatment

The growth differences between *X. italicum* and *G. uralensis* in mixture treatment were more significant than those in monoculture treatment. Under LF treatment, the height, crown width, stem diameter, SLA, root biomass, stem biomass, leaf biomass, and total biomass of *X. italicum* were 7.2-, 3.82-, 9.46-, 0.4-, 7.74-, 369.46-, 130.88-, and 56.86-fold higher,

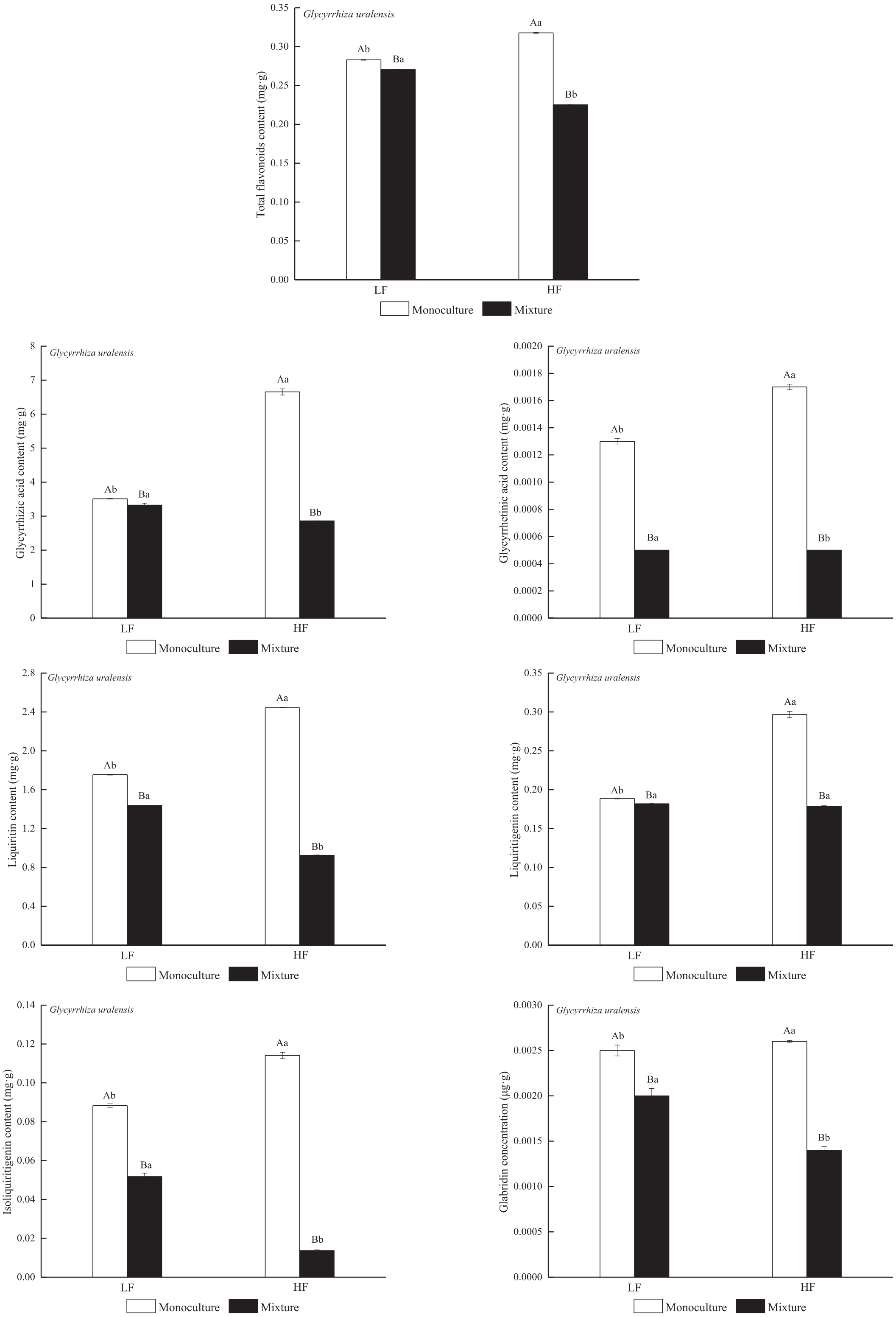

Fig. 4. Effects of cultivating patterns on secondary metabolite of *Glycyrrhiza uralensis* (mean±SE), the different capital letters means significant difference between monoculture and mixture treatments, the different lowercase letters means significant difference between LF (low-fertilizer) and HF (high-fertilizer) treatments.

Table 4. Comparison of the relative competitiveness of *Xanthium italicum* and *Glycyrrhiza uralensis*.

Competition parameters	LF		HF	
	Xanthium italicum	*Glycyrrhiza uralensis*	*Xanthium italicum*	*Glycyrrhiza uralensis*
RY	4.08±0.2 a**	0.31±0.01 a**	4.15±0.1a**	0.26±0.01 b**
RCI	-1.04±0.1 a**	0.85±0.03 a**	-1.07±0.05 a**	0.87±0.01 a**
A	3.77±0.12 a**	-3.77± 0.12 a**	3.89±0.11 a**	-3.89±0.11 a**

** represents significant differences between competition index and the hypothesis test value (i.e. *RY* is compared with 1, *RCI* and *A* are compared with 0, one-sample T test: *P*<0.01, n = 12). LF represents low-fertilizer treatment and HF represents high-fertilizer treatment. *RY*, *RCI* and *A* represent relative yield, relative competition intensity and aggressivity, respectively.

respectively, than those of *G. uralensis*. Meanwhile, the height, crown width, stem diameter, SLA, root biomass, stem biomass, leaf biomass, and total biomass of *X. italicum* were 7.53-, 3.23-, 10.42-, 0.3-, 10.35-, 515.45-, 82.06-, and 73.86-fold higher, respectively, for *X. italicum* under HF conditions than those of *G. uralensis* (Fig. 2).

Relative Competitiveness Comparison of *X. italicum* and *G. uralensis*

Under both fertility conditions, the *RY* of *X. italicum* was significantly greater than 1, while the *RY* of *G. uralensis* was less than 1, indicating that the intraspecific competition from *X. italicum* was greater than that from *G. uralensis*. The *RCI* of *X. italicum* was significantly less than 0, suggesting that the interplanting of *X. italicum* with *G. uralensis* was beneficial to the biomass accumulation of *X. italicum*. Meanwhile, the *RCI* of *G. uralensis* was between 0-1, indicating that *X. italicum* had a greater impact on *G. uralensis* and that *G. uralensis* exhibited weaker competitiveness in interplanting treatment. The *A* of *X. italicum* was significantly greater than 0, while that of *G. uralensis* was significantly less than 0, indicating that the competitive ability of *X. italicum* under the two fertility conditions was significantly greater than that of *G. uralensis* (Table 4).

Discussion

The competitive relationship between alien and native plants is one of the most important aspects of invasive ecology. Whether an alien plant can spread and cause harm to the environment is mainly determined by the biological characteristics of the species, with the intensity of interspecific competition being a crucial factor [26, 27]. While different species have various strategies for competing, plant size characteristics are the best parameters for predicting the relative competitiveness of plants, among which biomass is the most important parameter [28]. The results of the current study showed significant differences in the individual characteristics (size of different parts) of *X. italicum* and *G. uralensis*. Under LF and monoculture treatment, the height, crown width, and stem diameter of *X. italicum* were 3.25-, 0.87-, and 4.19-times as high, respectively, as those of *G. uralensis*, and its root biomass, stem biomass, leaf biomass, and total biomass were 0.48-, 19.19-, 4.51-, and 3.64-times as high, respectively, as that of *G. uralensis*. The height, crown width, and stem diameter of *X. italicum* in the HF treatment were 3.44-, 1.15-, and 3.49-times as high, respectively, as those of *G. uralensis*, and its root biomass, stem biomass, leaf biomass, and total biomass were 0.46-, 16.61-, 3.17-, and 3.98-times as high, respectively, as those of *G. uralensis*. When the two species were planted together, the differences in the body size of the two plants were greater. This means the alien cocklebur plants had taller stems, more developed branches, larger leaf areas, and more developed root systems than those of the liquorice plants. This may be an important reason for the alien plants being more competitive in utilizing light, water, and mineral elements than the liquorice plants [13, 29]. In addition, we found that *X. italicum* had a lower *SLA* than *G. uralensis*. It has been reported that low *SLA* is more conducive to plant growth [30], which means *X. italicum* had more favourable growth characteristics than *G. uralensis* in the case of mixture treatment.

Under monoculture conditions, the root biomass of *X. italicum* was only half of that of *G. uralensis*, and when the two species were planted together, the root biomass of the Italian cocklebur under LF and HF treatment was 7.74- and 10.35-times as high, respectively, as that of liquorice. This shows that the Italian cocklebur, under competitive conditions, had a well-developed root system, giving it an overwhelming advantage in the competition for limited water and inorganic nutrients in the soil. Furthermore, the well-developed leaves provided a larger photosynthetic area for *X. italicum* than the leaves of *G. uralensis*, which helped the invasive plant to produce more photosynthetic products and allowed it to develop larger vegetative organs than those of liquorice, thereby improving its invasiveness [31]. When interplanted with *G. uralensis* under LF and HF treatment, the stems of *X. italicum* reached 370- and 516-times, respectively, the biomass of *G. uralensis* stems. This showed that

X. italicum had obvious advantages over *G. uralensis* in occupying the above-ground space, resulting in unbalanced competition between the alien and native plants [32]. Taller stems, more developed branches, and larger leaf areas also meant that *X. italicum* could intercept more canopy light, which put the heliophilous plant *G. uralensis* in the lower layer with greater shade. This limited the accumulation of photosynthetic products in liquorice and severely inhibited its growth. Thus, the ability of *X. italicum* to compete for the utilization of environmental resources was improved, which acted an advantage. This is similar to the invasion strategy of the invasive plant *Impatiens balsamina* [33]. Under mixture treatment with both LF and HF conditions, the fruit yield of *X. italicum* was increased by 38% and 51%, respectively, compared to that under monoculture treatment. Therefore, *X. italicum* was able to produce more seeds than *G. uralensis* in the liquorice farmlands. Excellent overwintering performance [11] and high seed production by *X. italicum* significantly improved its sexual reproduction ability. Accordingly, more individual *X. italicum* plants will appear on the farmland during the next growing season. The expected increase in the abundance of *X. italicum* would in turn intensify the threats of competition by the alien plant and further suppress the growth of *G. uralensis*.

The competitive advantage of invasive plants over native plants for resources seriously affects the growth of the local plants [34]. The results of our study demonstrated that the growth of *G. uralensis* under interplanted conditions was significantly inhibited by strong competition from *X. italicum*. The stems and leaves of *G. uralensis* are a high-quality forage grass, which have extremely high value in improving the growth performance of cattle and sheep [18]. The results of the current study showed that the accumulation of stem and leaf biomass was extremely decreased under interplanted treatment, suggesting that the invasion of *X. italicum* will seriously reduce the production of *G. uralensis* as a forage source. Compared with that under monoculture treatment, the root biomass of *G. uralensis* under mixture treatment with LF and HF conditions was reduced by 84% and 88%, respectively. The root system is the medicinal organ of the liquorice plants, and a significant reduction in roots biomass means a correspondingly significant decrease in the yield of medicinal materials.

Plant competition studies focus mainly on changes in plant morphology and biomass, and usually ignore changes in the content of plant compounds, which is an important aspect because companion species may also have an impact on the accumulation of plant compounds [35]. In this study, we found that the content of seven medicinal compounds in liquorice was significantly reduced when liquorice was interplanted with the Italian cocklebur compared to that during the monoculture treatment. Furthermore, the inhibitory competitive effects on liquorice were significantly enhanced with increased levels of soil fertility. Under HF conditions, the root biomass, stem biomass, leaf biomass, and the content of total flavonoids, glycyrrhizic acid, glycyrrhetinic acid, liquiritin, liquiritigenin, isoliquiritigenin, and glabridin decreased by 88%, 93%, 89%, 29%, 57%, 71%, 62%, 40%, 88%, and 46%, respectively. This indicates that *X. italicum* not only led to a substantial reduction in the production of *G. uralensis* in terms of medicinal material, but also significantly decreased the accumulation of secondary plant metabolites and reduced the content of each compound in individual plants.

Meanwhile, *G. uralensis* is a clonal plant [36], and its rhizome not produces medicinal materials, but also plays a role in asexual reproduction. The results of our study showed that rhizome development of *G. uralensis* was completely inhibited when coexisting with *X. italicum*. The inhibition of rhizome development not only has a direct impact on the yield of medicinal materials, reduction in the number of asexual ramets also affects the actual population density of the liquorice plant and the total area of photosynthetic organs in the farmland. This in turn affects the yields of forage material (stems and leaves) and the production of the medicinal materials (roots and rhizomes). We found that when interplanted with *X. italicum*, the development of nodules on the root system of *G. uralensis* was also significantly inhibited. Under mixture treatment with LF and HF conditions, the number of nodules was reduced by 81% and 95%, respectively, compared to that under monocultured treatment. Rhizobia have significant nitrogen-fixation ability [37]. The lack of rhizobium usually has a significantly greater impact on legumes than on other species [38], and a decrease in the number of rhizobia is bound to have a negative impact on the nitrogen level in the soil of the liquorice farmland.

The *RY*, *RCI*, and *A* values of plants are important indicators for explaining the competitive relationship between species [24]. This study showed that the intraspecific competition pressure from *X. italicum* was greater than that from *G. uralensis*. This means that the competitive ability of *X. italicum* was significantly greater than that of *G. uralensis*. Therefore, in the coexisting community of *X. italicum* and *G. uralensis*, *X. italicum* had an overwhelming advantage in the competition for various resources. With increased soil nutrient resources, the competitive advantage of invasive plants is even more significant. Liu et al. (2019) also found that high nitrogen content is beneficial for invasive plants [39], as it is an advantage against native plants, which is consistent with the results from our current study.

X. italicum has become an invasive weed worldwide [40]. Because wild *G. uralensis* has a wide range of habitats and is widely distributed in China, Russia, and Central Asian countries, the invasion of *X. italicum* will not only inhibit the growth, development, and reproduction of *G. uralensis* in farmland environments, but may also adversely affect the growth and reproduction of *G. uralensis* in natural environment. The invasion of *X. italicum* will disrupt the ecological balance of these environments and pose a serious threat to the local biodiversity.

Conclusions

In this study, replacement series experiments were conducted. Specifically, we designed a plant competition experiment to verify the invasion risk of the plant *X. italicum* to *G. uralensis* in farmland environments with different fertility levels, and analysed the effect of *X. italicum* on the growth, reproduction, biomass, and accumulation of secondary metabolites of *G. uralensis*. The results showed that the competitive ability of the invasive plant *X. italicum* was greater than that of *G. uralensis* under two levels of soil fertility, with the competitive advantage being more significant with increased soil fertility. The interspecific competition of *X. italicum* significantly inhibited *G. uralensis* stem and leaf growth, and reduced the plant height and crown width of *G. uralensis*, which strongly weakened its ability to capture light resources. Furthermore, *X. italicum* significantly inhibited biomass accumulation in the above-ground portion of *G. uralensis*, which seriously reduced its forage yield. The interspecific competition from *X. italicum* also significantly inhibited the development of *G. uralensis* roots and rhizomes, and the biomass and content of secondary metabolites in the roots and rhizomes was significantly reduced. This has a serious negative impact on the yield and quality of medicinal materials of the plant. Importantly, *X. italicum* will not only able to affect the growth, development, and reproduction of *G. uralensis* in farmland environments, but may competitively inhibit the growth and reproduction of *G. uralensis* in natural environment communities, thereby destroying ecological balance and posing a serious threat to the local biodiversity. While the pot experiments were used to simulate the liquorice farmland environment, only a small number of environmental factors were considered. The number of factors actually affecting the competition between *X. italicum* and *G. uralensis* in the real liquorice farmland environment is much higher. Therefore, it will be necessary to design competition experiments directly in the liquorice farmland environment in order to obtain more reliable results. While the current study provides important insights into the competitive inhibitory effect of *X. italicum* on *G. uralensis*, the mechanism behind this inhibitory effect needs to be further studied.

Acknowledgements

This study was supported by Grants from Science and Technology Development Special Project of Xinjiang Production and Construction Corps(2018AB012). We would like to thank the Analysis and Testing Center of Shihezi University for the technical assistance. We would also like to thank anonymous reviewers for their valuable suggestions to improve the manuscript.

Conflict of Interest

There are no conflicts of interest to declare.

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