Biological Control 139 (2019) 104093

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Biological control of *Solidago canadensis* using a bioherbicide isolate of *Sclerotium rolfsii* SC64 increased the biodiversity in invaded habitats

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ARTICLE INFO

Keywords:
Solidago canadensis
Biological control
Bioherbicide
Biodiversity
Sclerotium rolfsii

ABSTRACT

Solidago canadensis caused serious threaten to the local ecosystem. Herbicide-dependent control has caused new ecological consequences, such as biodiversity loss and environmental pollution. Biological control may mitigate such negative ecological impacts; but until now, this approach has not been applied to control *S. canadensis*. In our previous studies, strain SC64 of the fungus *Sclerotium rolfsii* held the potential of development as a bioherbicide to control *S. canadensis*. However, the effects of this practice on the plant community structure and biodiversity in invaded habitats have not been investigated. In this study, we selected four *S. canadensis*-seriously invaded habitats, in which treatments of SC64-based bioherbicide after plowing and chemical herbicide (glyphosate) were applied in each site, while no treatments was used as control. The weed control effect, the local weed community structure and biodiversity were monitored after treatment. The results showed that at 180 days after treatment, the bioherbicide treatment produced 89.61% weed control on average in each test site, while the chemical herbicide caused 70.06% weed control on average. In the bioherbicide treatment, significant increases in the total number of local weed species were observed, the importance value (IV) index of *S. canadensis* decreased by 70.5 and 67.7% in the spring and autumn, respectively (refer to 40.8 and 37.0% in chemical herbicide treatment). Our results also showed that bioherbicide treatment caused significant increases in ecological significance, i.e., richness, diversity and evenness, the average increase rates were significantly higher than those in the chemical herbicide treatment. In conclusion, compared with chemical herbicides, the SC64-based bioherbicide significantly improved the weed community structure and dramatically increased the biodiversity in habitats invaded by *S. canadensis*.

1. Introduction

Solidago canadensis is an herbaceous perennial plant of the Asteracea family. The plant is native to North America and has successfully invaded central and Western Europe, most of Asia, Australia, New Zealand, and other regions. To date, the species has become one of the most destructive and widespread invasive species in China (Lu et al., 2007; Weber, 2001). *S. canadensis* is a highly aggressive plant that presents rapid and profuse germination, fast growth, and high reproductivity (Banta et al., 2008; Dong et al., 2006a; Weber, 1997). In particular, the vigorous vegetative reproduction capability (clonal growth) of the plant is known to make the species highly invasive (Nuzzo et al., 2009). The invasion of *S. canadensis* has resulted in serious threats to native ecosystems; it has changed the structures and/or functions of the invaded ecosystems and has even caused extinction of native species under serious circumstances (Chen et al., 2013; Dong et al., 2015; Guo, 2005). Chen et al. (2013) conducted a comparative analysis of the weed communities between the habitats before and after introduced *S. canadensis*. This work has shown that the invasion of *S. canadensis* seriously decreased number of local weed species, and the compositions of the invaded weed communities were similar to those in the native range of *S. canadensis* (Chen et al., 2013). At present, *S. canadensis* poses a serious threat to the diversity and/or abundance of native weeds and to agricultural productivity in China, and has been recognized as an exotic malignant weed by the Chinese government (Abhilasha et al., 2008; Yang et al., 2008; Zhao et al., 2015).

At present, *S. canadensis* is mainly controlled by herbicides such as glyphosate, fluroxypyr, 2,4-D, and picloram through post emergence before the plant flowers (Guo et al., 2009; Shen et al., 2005). Although this method can control the aboveground parts of *S. canadensis* and

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https://doi.org/10.1016/j.biocontrol.2019.104093
Received 31 May 2019; Received in revised form 16 August 2019; Accepted 2 September 2019
Available online 03 September 2019

effectively reduce the seed-setting, it has little effect on the rhizome. In general, the rhizome of *S. canadensis* will regrow the plantlet through vegetative propagation shortly after the weed control is applied, which indicates that herbicides are a temporary solution to the problem (Guo et al., 2009). Moreover, because glyphosate is a non-selective, systemic herbicide, heavy application of a large amount of glyphosate can cause non-target effects and environmental pollution. The community structure and biodiversity of the invaded habitat are at risk to be destroyed after the chemical control of *S. canadensis*. Results from several studies suggest that long-term herbicides strategies led to a reduction in both species richness (Rotchés-Ribalta et al., 2016) and evenness (Mncube and Mloza Banda, 2018), as well as a decrease in weed diversity (García-Ruiz et al., 2018). An ideal approach to controlling *S. canadensis* needs to consider the restoration of biodiversity while ensuring the control effect.

Biological control of weeds by using plant pathogens has gained general acceptance as a practical, safe, and environmentally beneficial weed management method that is applicable to diverse agroecosystems (Shabana et al., 2003a,b; Shabana, 2005; Shabana et al., 2010). The potential of using alternative bioherbicides in place of synthetic herbicides has been generally accepted in agricultural, environmental and other fields (Ash et al., 2010; Boyette et al., 2014a; Boyette et al., 2014b). In the 1980s, *Puccinia chondrillina* was used in the Australian biocontrol program for *Chondrilla juncea*, which is the first successful example of using a pathogen to control invaded weeds (Kinane and Oliver, 2003). To date, more than 20 bioherbicides have been officially registered worldwide to control weeds, and at least 15 new classical biocontrol agents have been introduced (Charudattan, 2001). The fungus *Sclerotium rolfsii* isolate SC64 was originally isolated from diseased *S. canadensis* plants with destructive stem rot symptoms. Due to its high pathogenicity to *S. canadensis*, SC64 has been developed into a mycelial granule formulation for the management of *S. canadensis* (Tang et al., 2010). Previous studies showed that this bioherbicidal fungus presented a narrow host-range, stable pathogenicity and good environmental adaptability. Further studies have shown that a combination of plowing with SC64 bioherbicide technology presented high efficacy of *S. canadensis* control in the field trials and could almost inhibit the regrowth of the plantlet through the rhizome (Tang et al., 2013, 2011). However, the environmental adaptability of the technology has not been adequately tested. Even more importantly, it is necessary to assess the effects of this technology on the plant community structure and biodiversity in invaded habitats (Charudattan and Dinoor, 2000).

Therefore, we selected four different habitats that were seriously invaded by *S. canadensis*. Combinations of plowing with SC64-bioherbicide and chemical herbicide (glyphosate) were applied in each of the test sites. The objective of this study was in comparison with the herbicide treatment, to further evaluate the environmental adaptability for our SC64-bioherbicide technology in a field trial and investigate the effects of this biological control system on the plant community structure and biodiversity of the invaded habitat.

2. Materials and methods

2.1. Site description

The 4 test sites that were selected in this study were from two of the most common habitats: abandoned-field and artificial forest (Table 1). Each site had been invaded by *S. canadensis* for more than five years. Because each site had never been treated with herbicides, *S. canadensis* was growing at high densities, making it difficult to control. According to the habitat type, the local species of different sites exhibited great differences (Tables S1 and S2). The Luhe and Jiaxing sites were abandoned fields, the number of weed species and their densities were slightly higher than those of the other test sites, which were artificial forest. The species unique to the sites include *Bidens tripartita*,

Table 1
General situation of the test sites.

Experimental site	Location	Site area (m^2)	Habitat status	Average density of *S. Canadensis* (plants/m^2)	Experimental time	Time of weed diversity investigation
Lishui	31°61′N 118°91′E	6666.67	*Cinnamomum camphora* gap	80	2015.5.3	2016.3.23 and 2016.10.1
Luhe	32°30′N 118°81′E	8000	Abandoned-field	74	2015.5.5	2016.3.23 and 2016.10.1
Shanghai	30°97′N 121°12′E	3333.33	*Cinnamomum camphora* gap	86	2015.5.10	2016.3.24 and 2016.10.2
Jiaxing	30°68′N 120°67′E	3333.33	Abandoned-field	96	2015.5.13	2016.3.24 and 2016.10.2

Alopecurus japonicus, Alternanthera philoxeroides, *Ranunculus cantoniensis* and *Polygonum hydropiper*, which were hygrophytic weeds. The Lishui and the Shanghai sites were artificial *Cinnamomum camphora* forest, because of the shelter of the top trees, the number of weed species and their densities on these two sites were slightly lower.

2.2. Strain inoculum production

The pathogen *S. rolfsii* isolate SC64 was stored as stock cultures in sterile soil and then cultured on on potato dextrose agar (PDA: 200 g potato extract, 20 g D-glucose, 15 g agar, and water to make 1 L) for seeding cultures. A starter culture was produced by placing five agar plugs (5 mm diameter, which were cut from the actively growing margin of the PDA culture) into 500 ml potato dextrose broth in a 1 L Erlenmeyer flask (200 g potato extract, 20 g D-glucose, and water to make 1 L; pH 5.0). The starter culture was grown for 5 days on orbital shaker at 110 rpm and 28 °C before it was aseptically blended. The starter culture was then used to inoculate a solid substrate of cotton seed hull. A total of 400 g of the solid substrate and 100 ml of distilled water were placed in autoclavable bags (35-cm long, 30-cm wide). The bags were sterilized once (121 °C, 30 min) and allowed to cool before 100 ml of a blended starter culture of *S. rolfsii* was added. The starter culture was added using a sterile pipette and thoroughly mixed with the solid substrate under aseptic conditions. The bags were incubated at room temperature of approximately 25 °C in the dark for 6 days. Then, the inoculum was placed in trays and dried in the shade for approximately 24 h and used immediately for the experiments.

2.3. Experimental design

To avoid the influence of conditions in each test site, same experiments were conducted in four sites with same habitat (Lishui; Luhe; Shanghai; Jiaxing) (Table 1). In all four test sites, three evenly distributed experimental plots (each plots was 666.67 m^2) were set up (Fig. 1) that corresponded to three treatments:

1) Biological control of *S. canadensis* (bio): combination of plowing and the application of *S. rolfsii* inoculum (bioherbicide dosage: 80 kg/667 m^2, the content of effective components: 170 g/667 m^2) (Tang et al., 2013);

2) Chemical control of *S. canadensis* (ch): direct spraying glyphosate herbicide (herbicide dosage: 800 g/667 m^2, content of effective components of 49.2 g/667 m^2);

3) No treatment (ck).

2.4. Vegetation sampling and data collection

In each experimental plot of all four test sites, nine quadrats (1 × 1 m) were selected and were distributed in the form of "M" shape (zigzag) (Fig. 1). The density of *S. canadensis* in each quadrat was calculated as the number of weed stems per quadrat (plant/m^2) (Doucet et al., 1999) and the average density of *S. Canadensis* (AvD) in each experimental plot was obtained by calculating the mean value of the nine quadrats. The mortality of *S. canadensis* was determined 15, 30, 90 and 180 days after treatment by using the formula (Boyette et al., 2014a):

$$\text{Mortality}(\%) = \frac{\text{AvD in CK plot} - \text{AvD in experimental plot}}{\text{AvD in CK plot}}$$

To avoid the influence of seasons on our results, the weed species richness, the diversity indexes and evenness of the communities were investigated in spring and autumn in all four test sites (Table 1). We recorded the species names, abundance (density) (Doucet et al., 1999), height and individual species coverage of the plants (Tian et al., 1999). The plants were identified using "Flora of China" (Wu and Raven, 1994) and the "Chinese Virtual Herbarium" website (http://www.cvh.org.cn/). In every quadrat, 10 individual plants were randomly selected to measure the plant height (the vertical distance from the soil surface to the tip of the highest branch or longest leaf) for each species (if there were < 10 individuals present, all individuals were measured), and these measurements were used to calculate the average height. To measure the percentage of plant coverage, a 1 × 1 m metal frame with 100 cells (10 × 10 cm each) was placed above the canopy in each quadrat and all cells occupied by a particular plant species were summed. For each experimental plot, the average abundance, height, and individual species coverage of plants were obtained by calculating the average value of the nine quadrats. In addition, The longitude, latitude and elevation of every test site were recorded using a handheld GPS receiver (Garmin eTrex 20, Garmin International Incorporated Company, Kansas, USA).

2.5. Data analysis

Importance value (IV) is a comprehensive quantitative index that measures the growth and relative dominance of a species in a community. The relative IV was calculated using the following formula, which was slightly modified from (Mueller-Dombois and Ellenberg, 1974):

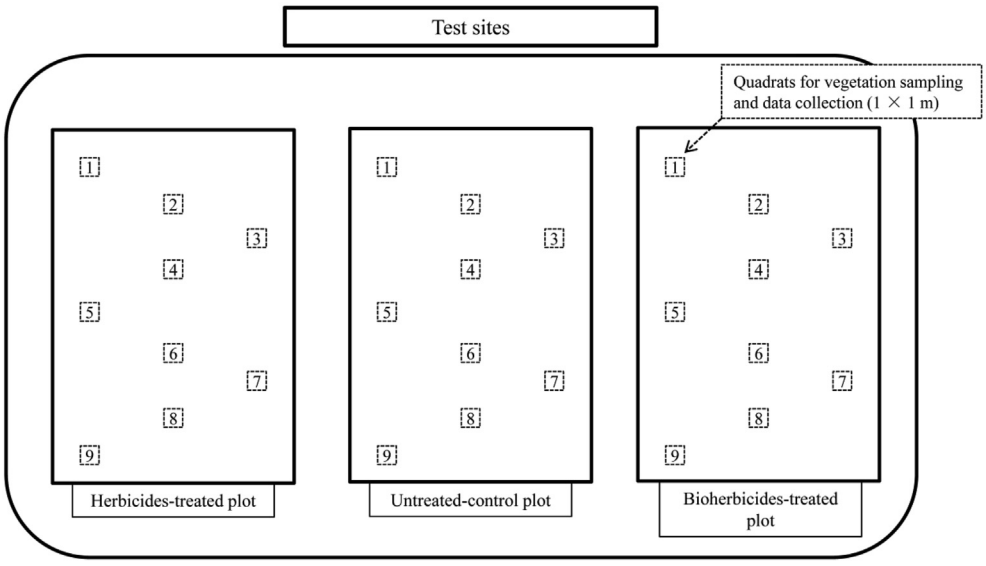

Fig. 1. The arrangement for three experimental plots in each test site showing nine survey quadrats (scattered boxes) in each experimental plot.

$$IV = \frac{\text{relative coverage} + \text{relative height} + \text{relative abundance}}{3}$$

where relative coverage, relative height, and relative abundance refer to the percentages of the cover, mean height, and abundance of one species over the sum of the cover, mean height, and total abundances of all species within a plot, respectively.

To measure the patterns of species diversity, four α species diversity indexes were employed, as follows (Derksen et al., 1995; Hurlbert, 1978; Izquierdo et al., 2009; Magurran, 1994; Nagendra, 2002):

Patrick richness index: $R = S$

where S is the number of species within a plot

Simpson diversity index: $\lambda = 1 - \Sigma P_i^{\ 2}$

where P_i is the proportion of individual numbers of the i species to the total individual number of each species in the quadrat. It is calculated from the formula as $P_i = n_i/N$ where N is the total individual number of each weed species and n_i is the individual number of the i species.

Shannon-Wiener diversity index: $H = -\Sigma P_i \ln (P_i)$

Pielou evenness index: $E = \frac{(-\sum Piln(Pi))}{\ln S}$

Microsoft Excel 2010 was used to calculate the statistics, and SPSS 16.0 software (SPSS Inc., Chicago, USA) was used to test for significant differences.

Statistical analysis was conducted using SPSS software (IBM Corp., Armonk, NY, USA for IBM SPSS Statistics (20.0)). One-way ANOVA was used to analyze the effect of treatment or test sites. Differences among means were tested with Duncan's test and significance was determined at $P < 0.05$ or $P < 0.01$. The figures were drawn by Microsoft Excel 2010.

3. Results

3.1. Weed control effect of different treatments

The effects of biological and chemical control on the mortality of *S. canadensis* 15, 30, 90 and 180 days after treatment are shown in Fig. 2. There was no significant difference between the efficacy of the two control methods 15 and 30 days after treatment. Among the four test sites, biological control resulted in 84.3 and 87.4% mortality of *S. canadensis* on average after 15 and 30 days, respectively, while chemical control resulted in 81.8 and 85.4% weed mortality, respectively. The weed suppressing effect of the herbicide significantly ($P < 0.05$) reduced 90 and 180 days after treatment (Fig. 2). Among the four test sites, *S. canadensis* mortality induced by the chemical herbicide resulted in 76.6 and 70.1 on average after 90 and 180 days, respectively. However, the herbicidal effect of the bioherbicide remained stable; resulting weed mortality in 92.1 and 89.6 on average after 90 and 180 days, respectively. Thus, the average weed suppression effect of the bioherbicide was significantly higher than that of the chemical herbicide ($P < 0.01$) both 90 and 180 days after treatment among all test sites (Fig. 2).

3.2. Impact of SC64-based bioherbicide on the weed species composition in S. canadensis-invaded habitats

Both biological and chemical control effectively reduced the population density of the target weed *S. canadensis*. Compared to the untreated-control plots, biological control decreased the density of *S. canadensis* by 90.1 and 79.1% in the spring and autumn, respectively ($P < 0.05$). However, chemical control decreased the density by only 73.4 and 66.4% (no significant) in the spring and autumn, respectively (Fig. 3). Despite both treatments have reduced the density of the target weed, the two treatments had different impacts on the weed species composition. Increased numbers of non-target weed species were observed in the bioherbicide-treated plots. Total list of weed species observed in this study is shown in Tables (S1 and S2). To evaluate the

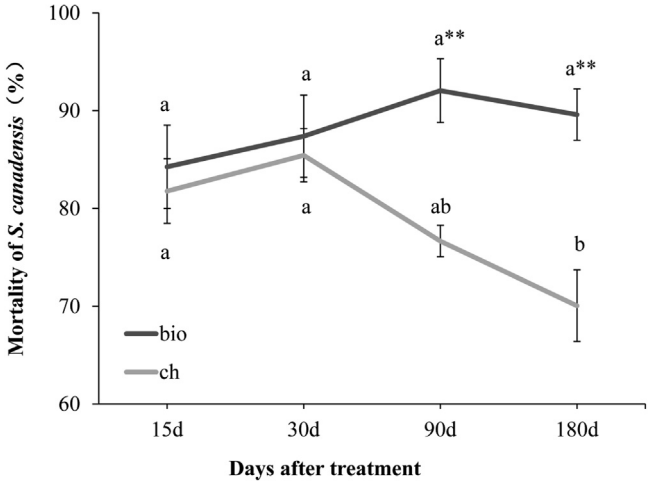

Fig. 2. Effect of biological and chemical herbicides on mortality of *S. canadensis* 15, 30, 90, and 180 days after treatment. Note: Data represent means ± SD (n = 4 test sites). (bio: Biological control; ch: Chemical control). Different lower-case letters indicate significant differences among times within a treatment ($P < 0.05$). Double asterisks indicate significant differences between treatments at a time ($P < 0.01$).

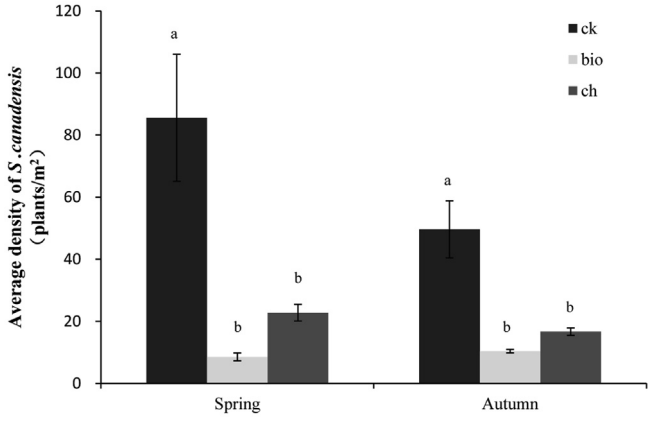

Fig. 3. Average densities of *S. canadensis* obtained from the investigation of weed diversity in the spring and autumn. Different lower-case letters indicate significant differences among the treatments ($P < 0.01$). Data represent means ± SD (n = 9 replications). (ck: untreated-control plots; bio: bioherbicide-treated plots; ch: chemical herbicide-treated plots).

ecological impact of the SC64-based bioherbicide, the average numbers of weed families, genera, and species were calculated as the mean value among the four test sites in the spring and autumn. In the spring, 26 plant species from 13 families and 24 genera were evenly recorded in the bioherbicide-treated experimental plots, while 15 weed species from 9 families and 14 genera were observed in the untreated-control plots ($P < 0.05$ for the difference among the treatments) (Fig. 4a). However, chemical herbicide treatment slightly increased (no significant) the total number of weed species compared to the untreated-control; 19 species of plants from 9 families and 18 genera were found in the herbicide-treated plots. Compared to the untreated-control, populations of the weed species *Hemistepta lyrate*, *Bothriospermum chinense*, *Salvia plebeian*, and *Polygonum lapathifolium* only occurred in bioherbicide-treated plots. Similar results were observed in the autumn survey. Compared to the untreated-control plots (9 species belonging to 6 families and 9 genera), weed species were increased twofold ($P < 0.05$) in the bioherbicide-treated plots (18 weed species from 10 families and 18 genera), while there was slight increase (no significant) of the number of species after herbicide application (13 species, 8 families, 13 genera) (Fig. 4a). In order to determine the effects of

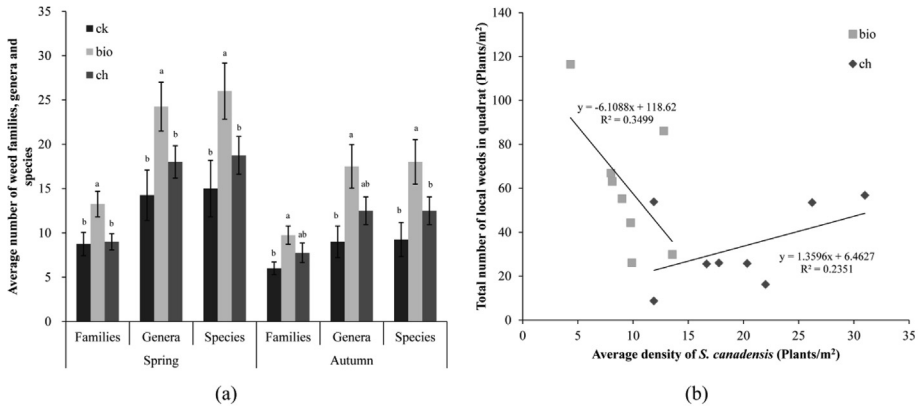

Fig. 4. Impact of different treatments on weed species composition. (a) Average numbers of weed families, genera, and species among four test sites in the spring and autumn. Different lower-case letters indicate significant differences among the treatments ($P < 0.05$). Data represent means ± SD (n = 4 test sites). (b) Regression analysis between density of *S. canadensis* and the total number of local weed species in 1 × 1 m quadrat from different experimental plots. (ck: untreated-control plots; bio: bioherbicide-treated plots; ch: chemical herbicide-treated plots).

different control technologies on the community structure of the habitat, a linear regression analysis was conducted on the density of *S. canadensis* and the total number of local weed species (per 1 × 1 m quadrat) in the SC64-based bioherbicide and chemical herbicide experimental plots (Fig. 4b). The results showed that the total number of local weed species was negatively correlated with the density of the target weed *S. canadensis* after bioherbicide treatment, while it was positively correlated after the chemical herbicide treatment. Overall, these results indicate that bioherbicide treatment significantly enriched the plant species composition in the habitats where the number of plant species had declined due to *S. canadensis* invasion.

3.3. Impact of SC64-based bioherbicide on the weed community structure in S. canadensis-invaded habitats

In the untreated-control plots of all four test sites, *S. canadensis* had the largest IV index (IV = 35.2 and 46.8 in the spring and autumn, respectively) and dominated the plant community. After bioherbicide application, the IV index of *S. canadensis* decreased by 70.5 and 67.7% in the spring and autumn, respectively ($P < 0.01$) (Fig. 5a). In addition, the dominant species changed in all four test sites (Tables S1 and S2). For instance, at the Lishui site, *S. canadensis* co-dominated with *Vicia tetrasperma* and *Daucus carota* in the spring and co-dominated with *Conyza canadensis* in the autumn. The Luhe site was dominated by *Alternanthera philoxeroides* in the spring and co-dominated by *Alternanthera philoxeroides*, *Panicum bisulcatum*, and *S. canadensis* in the autumn. At the Shanghai site, *S. canadensis* was co-dominated with *Eclipta prostrata*, *Conyza canadensis*, *Veronica persica*, and *Cerastium arvense* in the spring and co-dominated with *Acalypha australis* in the autumn. At the Jiaxing site, *S. canadensis* was co-dominated with *Ranunculus chinensis*, *Cardamine hirsuta*, and *Geranium carolinianum* in the spring and co-dominated with *Digitaria sanguinalis* and *Leptochloa chinensis* in the autumn. After the chemical herbicide application, the IV of *S. canadensis* decreased only by 40.8 and 37.0% in the spring and autumn, respectively ($P < 0.01$). However, *S. canadensis* was still the dominant species almost in all of the test sites in both the spring and autumn (except for the Shanghai site in the spring). The linear regression analysis of the density and the IV index of *S. canadensis* revealed that the density of *S. canadensis* was positively correlated with the IV index of *S. canadensis* after bioherbicide treatment, but there was no significant relationship between the two variations after the herbicide treatment (Fig. 5b). This result indicated that biological control could effectively reduce the population density as well as the dominance of *S. canadensis* in the community. As a result, the dominance of other species in the community was restored, and the weed community structure improved significantly. On the contrary, chemical control has suppressed *S. canadensis* but also killed other plant species in the community. Moreover, *S. canadensis* regrew and quickly became dominant after the chemical herbicide treatment.

3.4. Impact of SC64-based bioherbicide on the weed diversity in S. canadensis-invaded habitats

In all four test sites, compared with the untreated-control plots (CK), the weed species richness, the diversity indexes, and evenness of the communities increased significantly after biological control of *S. canadensis* in both spring and autumn (Fig. 6). Among the four test sites, the species richness index (S) increased by 72.4 and 138.1% in the spring and autumn, respectively. The Simpson diversity index (D) increased by 133.8 and 589.5% in the spring and autumn, respectively. The Shannon-Wiener diversity index (H) increased by 145.9 and 497.7% in the spring and autumn, respectively. And the Pielou evenness index (J) increased by by 83.6and 162.2% in the spring and autumn, respectively. All of the differences were significant ($P < 0.05$). Taken together, these results suggest that the biological control of *S. canadensis* enhances the weed species diversity and evenness of the

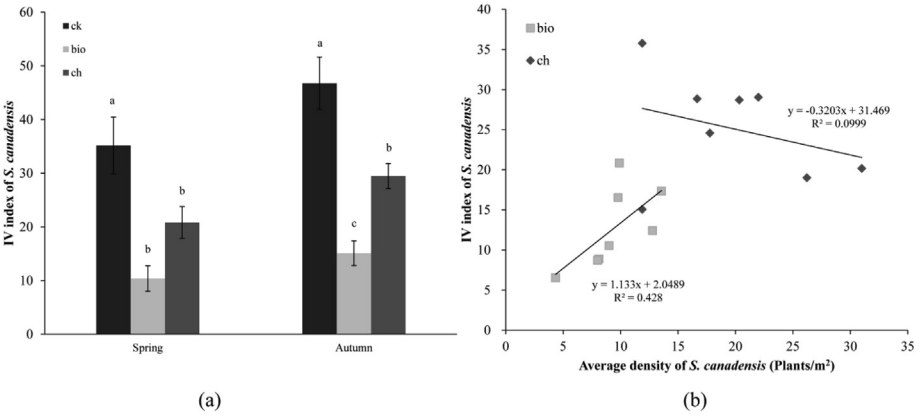

Fig. 5. Impact of different control treatments on weed community structure. (a) Average IV index of *S. canadensis* obtained from the investigation of weed diversity among 4 four sample test sites in spring and autumn. Different lower-case letters indicate significant differences among the treatments ($P < 0.01$). Data represent means ± SD (n = test sites). (b) Regression analysis between density and the IV index of *S. canadensis* from different experimental plots. (ck: untreated-control plots; bio: bioherbicide-treated plots; ch: chemical herbicide-treated plots).

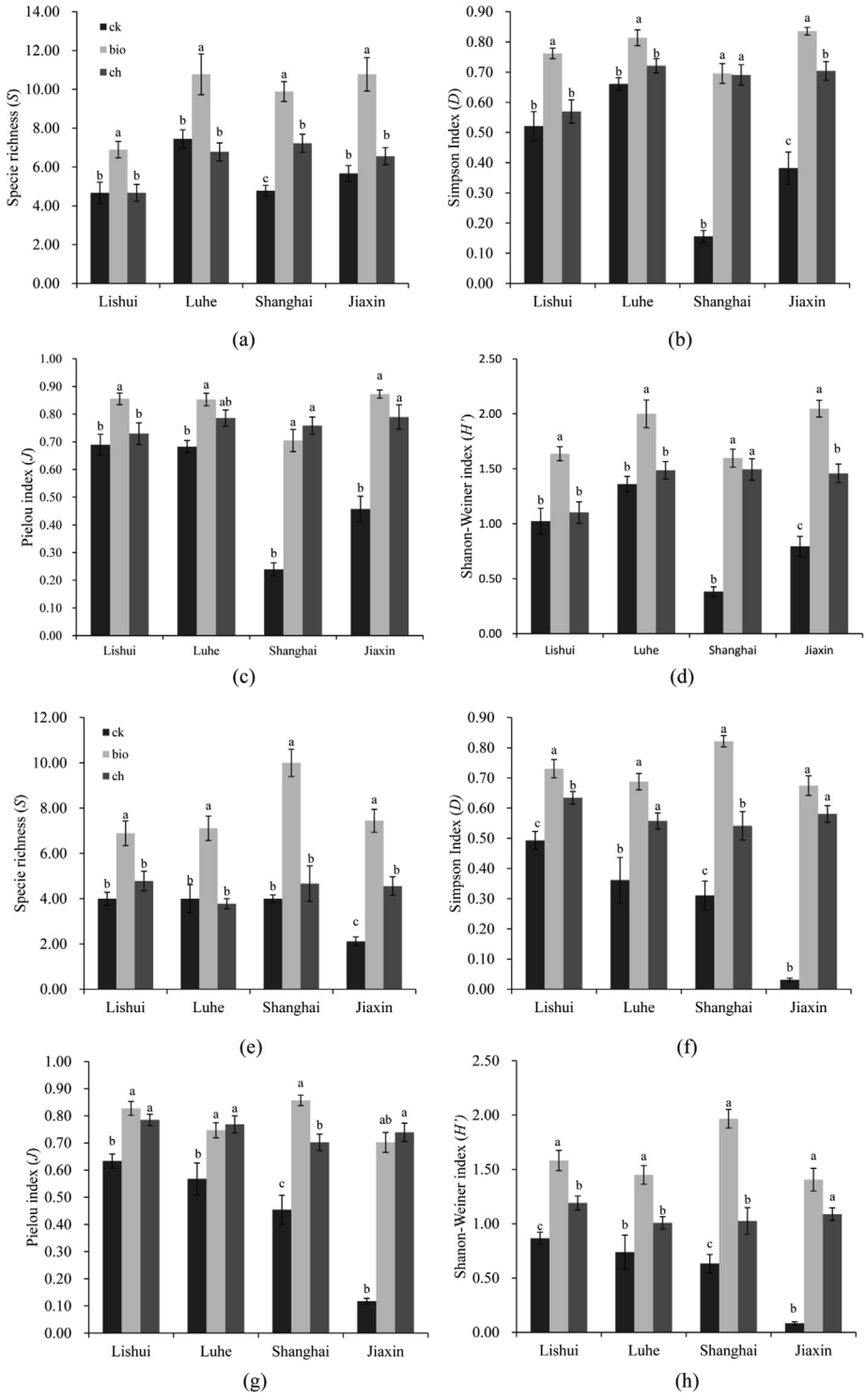

Fig. 6. Impacts of different control treatments against *S. canadensis* on weed species diversity indexes and evenness of the communities in different test plots sites. (a), (b), (c) and (d): survey data in spring; (e), (f), (g) and (h): survey data in autumn. Different lower-case letter (s) indicate significant differences among the treatments ($P < 0.05$). Data represent means ± SD (n = 9 replicates). (ck: untreated-control plots; bio: bioherbicide-treated plots; ch: chemical herbicide-treated plots).

communities in invaded habitats regardless of season or habitat. However, compared with the untreated-control plots (CK), the effects of chemical control on the four indexes of weed diversity and evenness of the communities varied according to the test site or season. At the Lishui site, the four weed community diversity indexes seamed to increase in autumn ($P < 0.05$) but did not change in spring. At the Luhe site, these indexes did no change after chemical control (except for the Simpson diversity index (*D*) and The Pielou evenness index (*J*) in autumn). At Shanghai and Jiaxing sites, these indexes increased significantly in both spring and autumn ($P < 0.05$). Moreover, compared with the bioherbicide-treated plots (bio), the four weed community diversity indexes in most test sites were significantly higher than in herbicide-treated plots (ch) in both spring and autumn ($P < 0.05$). Overall, these results indicate that, although chemical control could improve weed species diversity indexes and evenness of the communities to a certain extent, it is far less effective than biological control in

restoring weed communities.

4. Discussion

Previous successful biological control trials using *Sclerotium rolfsii* isolate SC64 were only conducted at one site (Tang et al., 2011). In this study, four test sites had been selected including two of the most common *S. canadensis*-invaded habitats (abandoned-fields and artificial forest). Our result indicates that the biological control can be applied to various *S. canadensis* invaded habitats, and demonstrated an excellent weed-suppression effect in all sites. The weed control effect of SC64 bioherbicide showed a wide range of adaptability to different habitats.

In this study, the weed-suppression effect of glyphosate was decreased significantly over time after treatment and the sustainability of its effect was unsatisfactory. These results are likely to be related to the ability of *S. canadensis* to regenerate vegetative growth from small underground fragments of stem and rhizome after the foliage is damaged. It is therefore difficult to eradicate *S. canadensis* because the traditional chemical control cannot effectively destroy the underground parts of the weed plants (Dong et al., 2006b). *S. canadensis* can regenerate vegetatively from underground rhizomes, and quickly establish its dominant state and eventually lead to the failure of chemical control. However, the weed control effect of the bioherbicide was sustained and stable in all four test sites. Previous studies have shown that *S. rolfsii* SC64 can directly infect the rhizomes of *S. canadensis*, which causes severe rhizome rot and thus inhibits the vegetative regeneration (Tang et al., 2013). Therefore, *S. canadensis* can be completely eradicated by combining biological control with plowing.

The invasion of *S. canadensis* resulted in the homogenization of diverse communities that were all dominated by *S. canadensis* (Chen et al., 2013). The purpose of the control was not only to reduce the *S. canadensis* population density but also to restore the weed species composition and community structure in the invaded habitat. In this study, the population density of *S. canadensis* was significantly reduced after chemical herbicide and bioherbicide treatments. Theoretically, the reduction in the population density of *S. canadensis* can release the space for other weed species and effectively increase the total number of species in the habitat. However, after chemical control, the total number of weed species and density of the local weeds were not effectively enhanced, and the herbicide-treated experimental plots in all four test sites were still remained dominated by *S. canadensis* and its IV index was the highest among all weed species in all sites. A possible explanation for this result might be that herbicide treatment could reduce the population density of *S. canadensis* and kill a large number of native weeds at the same time, leading to destroying the local weed community structure and damaging the environment. Even if the density of *S. canadensis* decreased significantly, the total number of local weed species could not be effectively restored and neither did the local weed community structure. On the contrary, most of the weed community structure was significantly improved after the application of biological control. The decrease of *S. canadensis* density after bioherbicide treatment could effectively enhance the total number of local weed species, which might significantly reduce the IV index of *S. canadensis* in the community and *S. canadensis* was then replaced by local weed species after bioherbicide treatment. Some sensitive species such as *Salvia plebeian* from Labiatae, *Stellaria media* from Caryophyllaceae and *Metaplexis japonica* from Asclepiadaceae were found with the local native species (Tables S1 and S2), which indicate that the weed species composition became increasingly complex. According to the differences among the habitats, there were different dominant weed populations in all bioherbicide-treated plots, and their IV indexes were significantly higher than that of *S. canadensis*. The results showed that the biological control was harmless to the native plants. When the population density of *S. canadensis* decreased, the total number and density of other species were effectively increased, and the dominance of the local weed species in the community structure were also restored.

Environmental selection pressure plays a pivotal role in the species composition and biodiversity of different habitats (Liu et al., 2005; Wu et al., 2006), which has a great impact on habitat restoration after *S. canadensis* control. Hence, in this study, we evaluated the effects of different control technologies on the biodiversity in four habitats. We found that bioherbicides could improve weed species diversity indexes and evenness of the communities in all four test sites. In contrast, the trends of the diversity indexes after chemical herbicide application were greatly fluctuated with test site and season. The diversity indexes after bioherbicide application were significantly higher than those of the sites that were treated with the herbicide. Further analysis revealed that although the weed community diversity indexes of some test sites (Shanghai and Jiaxing) increased significantly after herbicide application, the plant community structure at these sites was dominated by plants that accompanied *S. canadensis* (Chen et al., 2013) and common weeds which were resistant to chemical herbicides (Owen et al., 2007) (Tables S1 and S2). In fact, one of the potential risks of long-term chemical herbicide use is the increasing tolerance of some malignant weeds. In this study, the test site of Jiaxing was abandoned field where dominant species such as *Conyza canadensis*, *Alopecurus aequalis* and *Beckmannia syzigachne* were found in the fields after herbicide application (Tables S1 and S2), which are common farmland weeds (Mueller et al., 2003; Owen et al., 2007; Patzoldt et al., 2005). Many studies have reported the resistance of these weeds to chemical herbicides. After several years of farming, the weeds in the region are likely to be resistant to chemical herbicides. After chemical elimination of *S. canadensis*, the populations of these resistant weeds will be rapidly recovered. It is worth to know that population expansions of such resistant weeds are not beneficial to ecological restoration.

Based on the results of this study, we found that although chemical control could reduce the population density of the target weed *S. canadensis* to some extent, it would kill some of the non-target native weed species at the same time, resulting in the destruction of the local weed community structure, weed species diversity indexes and evenness of the community. Large areas of bare land were found in the aerial photographs (Figs. S1 and S2-C), indicating that the space freed after *S. canadensis* was eliminated could not be effectively occupied by native species. In contrast, the number and the density of local species increased after bioherbicide treatment, which could rapidly cover the ground surface (Figs. S1 and S2-B), at the same time, the local weed community structure can be restored effectively. What is more, bioherbicide treatment can also play an important role in the protection of local animals and other ecological communities and can reduce soil erosion.

CRediT authorship contribution statement

Yu Zhang: Conceptualization, Investigation, Methodology, Writing - original draft. **Xianghong Yang:** Data curation, Formal analysis, Investigation, Writing - review & editing. **Yibing Zhu:** Investigation. **Lingyan Li:** Resources. **Yali Zhang:** Resources. **Junping Li:** Investigation. **Xiaoling Song:** Funding acquisition. **Sheng Qiang:** Project administration, Supervision.

Acknowledgements

This work was founded by the National Natural funds for Distinguished Young Scholar [3140110504]; the Fundamental Funds for the Central Universities [KJQN201535] and the National Key Research and Development Program [2017YFC1200105].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2019.104093.

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