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RESEARCH ARTICLE

Biological control of the invasive alien weed *Solidago canadensis*: combining an indigenous fungal isolate of *Sclerotium rolfsii* SC64 with mechanical control

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Solidago canadensis L. is a major invasive weed that is highly tolerant to disturbances and difficult to control in China. In order to develop a rapid non-chemical control strategy for this weed in heterogeneous environments, we investigated different treatments including mechanical control (cutting and hoeing) and inoculation with an indigenous pathogen, *Sclerotium rolfsii* SC64, which was isolated from *S. canadensis* and applied by means of a solid formulation. Greenhouse and field trials were conducted to test how the control regimes (i.e. individual treatment methods, combination of these methods and different treatment timing) influence control efficiency. The fungal isolate *S. rolfsii* SC64 caused 70% plant mortality and fresh weight reduction of *S. canadensis* under 150 cm growth stage, and efficacy increased to 80% when the above-ground material was removed. However, the use of cutting, hoeing or treating with *S. rolfsii* SC64, on its own, did not provide sufficient control of *S. canadensis*. Cutting treatments performed in July and September only eliminated sexual reproduction of *S. canadensis*. Combination of cutting, hoeing and treating with isolate SC64 during the growing season in May, July and September was able to kill more than 90% of the ramets. This combination of methods not only eliminated sexual reproduction of *S. canadensis*, but also destroyed its underground stems and prevented its regrowth. Therefore, this integrated approach may provide an optima control strategy for *S. canadensis*.

Keywords: Canadian goldenrod; mycoherbicides; integrated weed management; *Sclerotium rolfsii*; *Solidago canadensis*

Introduction

Solidago canadensis L. (Canadian goldenrod, Asteraceae), a long-lived rhizomatous perennial plant native to North America, was intentionally introduced to Shanghai, China in 1935, as an ornamental, but has now become a major invasive species in four provinces of southeastern China and continues to spread northward and westward (Guo, 1995; Jin, Gu, Xiao, Chen, & Li, 2004; Li & Xie, 2002;). It is quite common around field borders, road-sides, gardens, orchards, undeveloped areas and even green spaces of some cities. Therefore, *S. canadensis* has been listed as a quarantine organism by the State Forestry Administration of the People's Republic of China. Under natural conditions, *S. canadensis* has great reproductive capacity through both seed production following insect pollination and clonal growth from

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underground stems (Figure 1). A mature *S. canadensis* plant may produce more than 20,000 seeds (Hartnett & Abrahamson, 1979), and supports an approximate 80% germination rate under ideal conditions (Walck, Baskin, & Baskin, 1998; Werner, 1979). Seeds are spread by wind or other dispersal mechanisms and readily establish new populations, if conditions are suitable. In addition, naturalised populations have great capacity to propagate clonally (i.e. produce ramets) via their underground stems. Agriculturally, *S. canadensis* populations in farmland compete for nutrients and space with crops, causing yield losses to agricultural production. Environmentally, weed populations have significantly reduced the abundance and diversity of plant communities that are native to China (Dong, Lu, Zhang, Chen, & Li, 2006).

In order to reduce the sexual reproduction of *S. canadensis*, two control measures have been implemented. Mechanical control by cutting, mowing and controlled fires could dramatically decrease weed population densities, but is only suitable for small plots and does not remove all the underground stems. The residual stems or the uprooted plants with flowering branches are still able to produce viable seeds and become a new source of invasion (Guo, Jiang, Fang, & Chen, 2009). Herbicides such as glyphosate, fluroxypyr and several systematic products such as 2, 4-D and picloram are applied by spraying before the plants produce flowers (Guo et al., 2009; Shen, Yao, Guan, Qian, & Ao, 2005; Weber, 2003). While herbicides may be useful in reducing the plants' above-ground biomass and inhibiting their flowering, they do not affect the subterranean stems from which regrowth can occur. Also, glyphosate is a non-selective, systemic herbicide that causes non-target effects and environmental pollution. The high number of phytophagous insect species found on *S. canadensis* in North America suggests that there is no shortage of possible biological control agents (Jobin, Schaffner, & Nentwig, 1996). There are several insects, such as *Epicauta pennsylvanica*, *Lopideam media* and *Lygus lineolaris* that feed on *S. canadensis* in its native range (Irwin, 1985). However, *S. canadensis* in its invasive range only possesses a fraction of the insect herbivores that are found within its

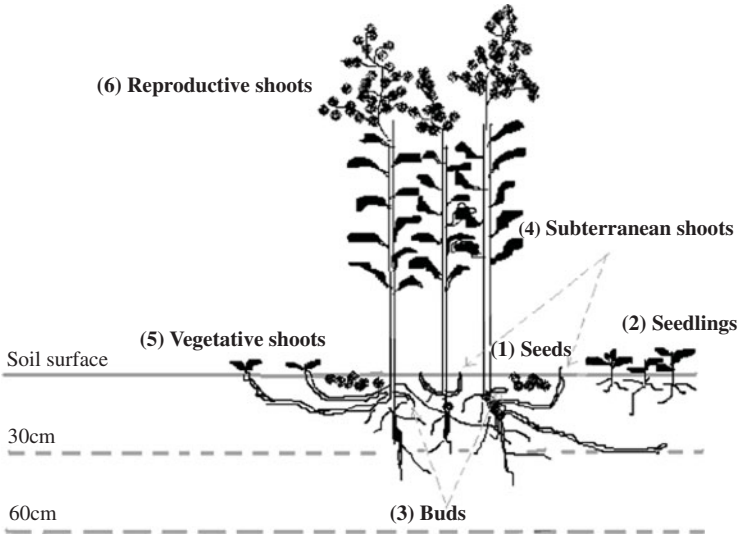

Figure 1. The six life-history classes of *S. canadensis* recognised in this study.

native range (van Kleunen & Schmid, 2003), and few fungal pathogens or bacteria suppress this invader. In Japan, *S. altissima* exhibits bacterial leaf spot caused by *Pseudomonas syringae* pv. *Solidagae* (Sato, Watanabe, & Sato, 2001), but there is no evidence that *S. canadensis* could be infected by this bacterium.

Biological control is often the last recourse for the management of weeds that have large negative impacts and cannot be controlled through other methods (Myers & Bazely, 2003). To our knowledge, biological control of *S. canadensis* has not been attempted in China, until an isolate of *Sclerotium rolfsii* was recently isolated from diseased *S. canadensis* plants with destructive stem rot symptoms, which have been sporadically occurring on populations in Nanjing city, in the Jiangsu province of China (Tang, Zhu, He, & Qiang, 2010). Due to its high pathogenicity on *S. canadensis*, the prospect to develop *S. rolfsii* isolate SC64 as a bioherbicide for this weed is now being considered.

The objective of this study was to examine: (1) the susceptibility of different growth stages of *S. canadensis* to *S. rolfsii* isolate SC64 in pot trials in a greenhouse; and (2) the control efficiency of different methods such as cutting, hoeing and inoculation with *S. rolfsii* isolate SC64, alone and in combination, and at different times in a growing season under field conditions and (3) the regeneration capability of cut stem sections of *S. canadensis*.

Materials and methods

Inoculum production

The pathogen *S. rolfsii* isolate SC64 was stored as stock cultures in sterile soil and grown on potato dextrose agar (PDA: 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 (potato extract, 20 g D-glucose and water to make 1 L, pH 5.0). The starter culture was grown for 7 days in orbital shake flasks at 110 rpm and 28°C before it was aseptically blended. The starter culture was then used to inoculate a solid substrate of mixed rice husk/bran (2:1, v/v). Three hundred grammes of the solid substrate and 100 ml of distilled water were placed in autoclavable bags (35-cm long, 30-cm wide). The bags were sterilised once (121°C, 30 min) and allowed to cool before adding 45 ml of blended starter culture of *S. rolfsii*. The starter culture was added using a sterile pipette and mixed thoroughly with the solid substrate under aseptic conditions. Bags were incubated at 28°C in the dark for 7 days. Then inoculum was placed in trays and dried in the shade for approximately 24 h and used immediately for the experiments. The control treatment was uncolonised, autoclaved solid substrate.

Plant growth stage and susceptibility

Seed of naturalised *S. canadensis* was collected from the field in the suburb of Nanjing, Jiangsu Province of China. Plants were grown in a sterile potting medium (mixed vegetable garden soil/coversoil, 4:1, v/v) in pots (25 cm diameter, 30 cm height). Twenty-four pots (20 plants per pot) per growth stage of *S. canadensis* (four different growth stages were carried out: 5–10 cm; 25–30 cm; 40–50 cm; 100–150 cm in height and 100–150 cm immediately treated after removal of the above-ground plant material)

were treated at rates of 60, 80, 100, 120 or 140 gm $^{-2}$ of inoculum with four replicates for each treatment dose. Fungus-infested substrate was directly scattered on the soil surface. The control treatment was inoculated with 100 gm $^{-2}$ uncolonised, autoclaved solid substrate. After inoculation, plants were moved to a greenhouse with natural light and a temperature of 25–35°C. The soil was maintained wet throughout the experiment by adding water to saucers placed under the pots. Mortality of the plants to the fungal isolates was evaluated relative to the untreated control pots. Surviving plants were excised at soil level, weighed and the percentage of biomass reduction was determined relative to the untreated control plants at 14 days after inoculation.

Field trials

The experimental site was located at Cangbomen, a suburb of Nanjing city (32°1′34.92″N 118°52′46.27″E). The site was used for vegetable production and had been invaded by *S. canadensis* for 7 years. The total invaded area was approximately 300 m^2, with a few native plants present. As a result of never having been treated, *S. canadensis* was growing in high density and mixed up with their residual stems, making it difficult to control. An area with well-distributed *S. canadensis* was selected and different treatments were conducted during 2009 and 2010, with additional trials at a second site (see below) conducted during 2011.

2009 trials

Two trials were conducted on 20 July by direct scattering of fungus-infested substrate onto natural undisturbed plants and regrowth of *S. canadensis* population. Some 40 m^2 of *S. canadensis* ramets were previously cut 5–10 cm from the ground with a scythe on 12 May and served as regrowth population. After cutting, the plots contained 120–140 regrowth ramets per square metre at an average height of 110 cm. The undisturbed plants were 170 cm high with 110–130 ramets per square metre. Each treatment plot was 2 m $^{-2}$ with four replicates, and four rates of fungus-infested substrate (80, 100, 120 and 140 gm $^{-2}$) were applied in a randomised complete block design. Blocks of 40 m $^{-2}$ treated with 100 gm $^{-2}$ uncolonised, autoclaved solid substrate were used as control. Percentage mortality was determined at 30 days after treatment in relation to the untreated control plot.

2010 trials

Trials with five treatments (untreated control, UT; cutting, C; cutting + *S. rolfsii* SC64, CSc; hoeing, H; cutting + hoeing + *S. rolfsii* SC64, CHSc) were set up on three separate occasions on 20 May 2010, 20 July and 19 September, using a randomised plot design of the 2009 field trial. The sites were selected by searching for dense areas of *S. canadensis* within the experimental field of Cangbomen. After cutting and hoeing (CH) roots up, fungus-infested substrate was applied immediately at a rate of 120 gm $^{-2}$.

The experimental area was separated by a 5 m boundary to serve as a buffer zone. Each plot was carefully monitored for *S. canadensis* ramets and numbers per square metre were recorded 30 days after treatment. The percentage of *S. canadensis* ramet mortality was determined by comparing the numbers of ramets in the treatment blocks to those in the untreated control blocks. The influence of these treatments on

flowering of *S. canadensis* plants of the mid-May trial was also determined by comparing the number of seeds per inflorescence in the treatment blocks to those in the untreated control blocks after five months.

2011 trials

A second field trial was conducted from August to October 2011 at Haizikou, another suburb of Nanjing city (32°01′25″N, 118°53′25″E), which had been invaded by *S. canadensis* for 5 years. A similar experimental design was used as in 2010, including the treatments, except that each treatment covered an area of 50 m^2 and was not replicated. Half of the plots of treatments C, CSc, CH and CHSc were covered with cut *S. canadensis* plants in order to compare the effect of covering on biocontrol efficacy. Surviving *S. canadensis* ramets were recorded using quadrats (1 m × 1 m) placed randomly at five locations in each plot at 30 days after inoculation and percentage mortality was determined in relation to the untreated plots.

Regeneration capability of stem sections

On September 2011, stem sections of *S. canadensis* at 110 cm growth stage were collected at different heights above the ground (0–10 cm; 5–15 cm; 10–20 cm; 15–25 cm; 20–30 cm above ground) from the suburb of Nanjing. The stem sections (each size with 20 sections) were placed in potting medium (25cm diameter pot) as described above. The regeneration process of stems was observed continuously and regeneration rate (i.e. number of plants with leaves and roots developed from a stem section) was recorded after 14 days.

Statistical analysis

The data were presented as means ± SEs. One-way ANOVA and paired samples *t*-tests were employed to test the differences between the means, and two-way ANOVA was employed to compare the effects of both dosage and growth stage using the procedures in the SPSS 13.0 statistical package (SPSS Corp.). Least significant difference (LSD) post-hoc tests were employed, where the overall differences were significant ($P < 0.05$). A four-parameter logistic equation was fitted to the plant mortality data and LD_{50} or LD_{90} (mycoherbicide dose required to inhibit growth by 50% or 90%) values were estimated for each growth stage of *S. canadensis*, using the following equation:

$$Y = \frac{C + (D - C)}{\left[1 + (x/x_0)^b\right]}$$

Results

Plant growth stage and susceptibility

All five application rates of *S. rolfsii* SC64 caused significant levels of mortality for all the growth stages tested and significantly reduced the fresh weight of the surviving plants in relation to the controls ($P < 0.05$) under greenhouse conditions (Table 1).

Table 1. Mean (±SE) percentage mortality of *S. canadensis* at different growth stages after inoculation with different concentrations of *S. rolfsii* SC64 in greenhouse trials.

		Efficacy (%)							
Growth stage of *S. canadensis*		Nil (control)	60 gm^{-2}	80 gm^{-2}	100 gm^{-2}	120 gm^{-2}	140 gm^{-2}	LD_{50}/gm^{-2}	LD_{90}/gm^{-2}
<10 cm	MR	0e	51.3±4.3d	65.5±2.5c	76.8±3.2b	82.3±2.4b	93.8±2.4a	57.5	133.1
	FW	0e	65.5±3.6d	70.6±3.2cd	75.2±2.6bc	79.6±3.7b	88.8±5.1a	30.0	157.9
20–30 cm	MR	0e	27.6±3.3d	48.7±3.9c	55.3±3.4bc	67.1±4.5b	82.9±2.5a	89.5	155.4
	FW	0d	21.6±4.6c	49.4±9.4b	55.4±4.8b	69.9±7.4ab	87.6±1.9a	89.8	148.0
50–60 cm	MR	0e	28.0±5.0d	41.5±5.3c	59.8±3.7b	64.6±3.7b	76.8±4.2a	89.8	195.4
	FW	0e	20.2±3.6d	39.5±3.4c	57.9±2.8b	63.3±2.8b	78.9±4.1a	93.0	202.5
100–150 cm	MR	0c	24.4±3.2b	29.5±6.7b	61.5±9.5a	66.7±9.5a	74.4±6.6a	93.8	257.0
	FW	0c	13.0±4.6c	25.8±3.5bc	49.8±16.7ab	51.1±11.3ab	67.0±6.7a	108.1	>500
100–150 cm (moved the above-ground materials)	MR	0e	40.7±7.3d	53.1±5.1cd	62.8±3.4bc	72.6±4.7ab	82.3±2.5a	75.9	158.8
	FW	0d	39.6±4.0c	60.9±5.5b	77.4±3.6ab	81.6±2.6a	86.2±2.9a	68.4	196.0

Note: Means within the same row followed by different letters are significantly different at $P < 0.05$ level according to LSD test.
MR = mortality rate (percentage); FW = percentage of fresh weight reduction.

The isolate killed between 24% and 51% of treated plants by 14 days after inoculation, and caused between 13% and 66% reduction in fresh weight when applied at 60 g·m^{-2}. When the inoculum was increased to 140 g·m^{-2}, mortality rate and fresh weight reduction increased from 74 to 94% and 67 to 89%, respectively. The extent of mortality and growth reduction in *S. canadensis* was thus dose-dependent. The estimated LC_{50} and LC_{90} value for percentage mortality increased from 58 to 94 gm^{-2} and 133 to 257 gm^{-2}, respectively, as *S. canadensis* plants grew higher. Results in two-way ANOVA indicted that there were statistically, interaction at the $P = 0.549$ and $P = 0.130$ level for percentage mortality and fresh weight reduction, respectively, but there was significant difference in interest in percentage mortality between dosage or growth stage ($P < 0.0005$) (data not shown). Removing the above-ground material and then treating with *S. rolfsii* SC64 improved control efficiency at the same growth stage (100–150 cm). However, the target weed *S. canadensis* was able to regrow, and while isolate SC64 may kill above-ground plant tissues, the rhizomes could still survive and produce new ramets.

2009 field trials

Fungal mycelia grew out of the solid substrate within 24 h of application on the moist ground surface and spread in the organic layer, affecting contacted stems, roots and leaves of the host plants. Symptoms first appeared on *S. canadensis* 5–7 days after treatment with the highest inoculum dose. The first instance of mortality also occurred in the same treatment after 10–15 days.

Only some plants of *S. canadensis* were observed to have died at 30 days after inoculation. Mortality of *S. canadensis* in the two trials, involving natural (undisturbed) and regrowth populations, was lower than 22% (Table 2). The level of mortality increased with inoculation dosage, with a maximum of 21.2% and 20.7% at 140 gm^{-2}, in the natural and regrowth populations, respectively.

2010 field trial

Mortality of *S. canadensis* plants treated in mid-May varied between the different mechanical and fungal treatments, with maximum plant mortality of 93% recorded by using a combination of cutting, hoeing and fungal inoculation (CHSc) and was significantly ($P < 0.05$) higher than all other treatments (Figure 2). Simply cutting the ramets of *S. canadensis* was not sufficient to kill them, because new ramets would

Table 2. Mean (±SE) percentage mortality of *S. canadensis* ramets, 30 days after inoculation with different concentrations of *S. rolfsii* SC64, by direct scattering in natural and regrowth populations.[a]

	Treatment/gm^{-2}				
	80	100	120	140	UT
Natural population	6.3 ± 1.6c	10.7 ± 2.0bc	17.1 ± 3.3ab	21.2 ± 1.3a	0d
Regrowth population	8.9 ± 1.6b	14.6 ± 3.1ab	16.4 ± 3.5ab	20.7 ± 2.4a	0c

[a]Means followed by the same lower case letters in the same row indicate no significant difference ($P < 0.05$).

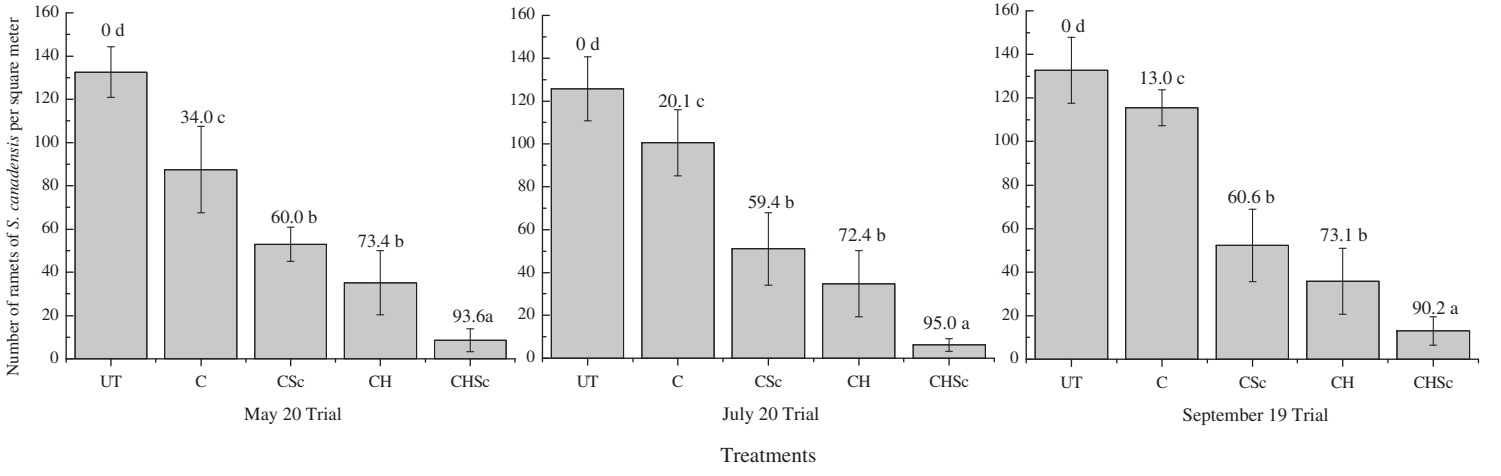

Figure 2. Effects of different control methods on *S. canadensis* at different times during the growing season. Bars indicate the mean (±SE) numbers of ramets/m^2 recorded 30 days after inoculation, while values above the bars indicate the percentage ramet reduction in relation to the control (UT). Bars designated by different lower case letters are significantly different ($P < 0.05$), where UT = untreated control; C = cutting; CSc = cutting + *S. rolfsii* SC64; CH = cutting + hoeing; CHSc = cutting + hoeing + *S. rolfsii* SC64.

sprout around the residual stems. Treatments cutting (C), cutting and fungal application (CSc) and cutting and hoeing (CP) killed 34%, 60% and 73% of *S. canadensis* ramets, respectively, in relation to the controls.

Results of trials initiated in mid-July and mid-September were similar to those initiated in mid-May (Figure 2). However, only the regrowth ramets in the mid-May trial were able to flower in October (Figures 3 and 4), with no inflorescences formed by plants regrowing after the mid-July and mid-September trials (picture not shown). This is probably because of insufficient time for vegetative growth. An assessment of flowering for the mid-May trial was recorded in mid-October, five months after inoculation. In relation to the untreated control, all four treatments significantly inhibited flowering (Figure 3). There was more damage to the roots, and fewer and smaller inflorescences appeared. Ramet in the plots subjected to a combination of cutting, hoeing and fungal inoculation (CPSc) rarely formed inflorescences.

2011 field trial

The results of the 2011 trial were similar to those of the 2010 trial. At 30 days after inoculation, plants exposed to a combination of cutting, hoeing and fungal inoculation (CHSc) displayed mortality in excess of 90% in relation to the controls (Figure 5). Treatments that were inoculated with fungus-infested substrate of *S. rolfsii* SC64 and then covered with cut plants displayed a relatively denser development of hyphae and provided better control efficacy, namely 95% compared with 91% in the uncovered plants. However, the mortality of plants subjected to CH and then covered with cut plants was lower than similarly treated but uncovered plants, probably because of lower water loss, which favoured regeneration. In

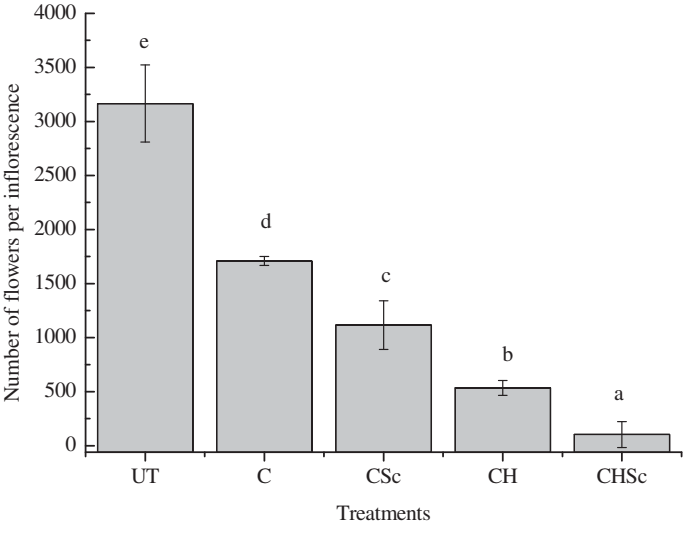

Figure 3. Number of flowers per inflorescence of *S. canadensis* plant treated with different control methods, where UT = untreated control; C = cutting; CSc = cutting + *S. rolfsii* SC64; CH = cutting + hoeing; CHSc = cutting + hoeing + *S. rolfsii* SC64. Bars designated by different lower letters are significantly different ($P < 0.05$).

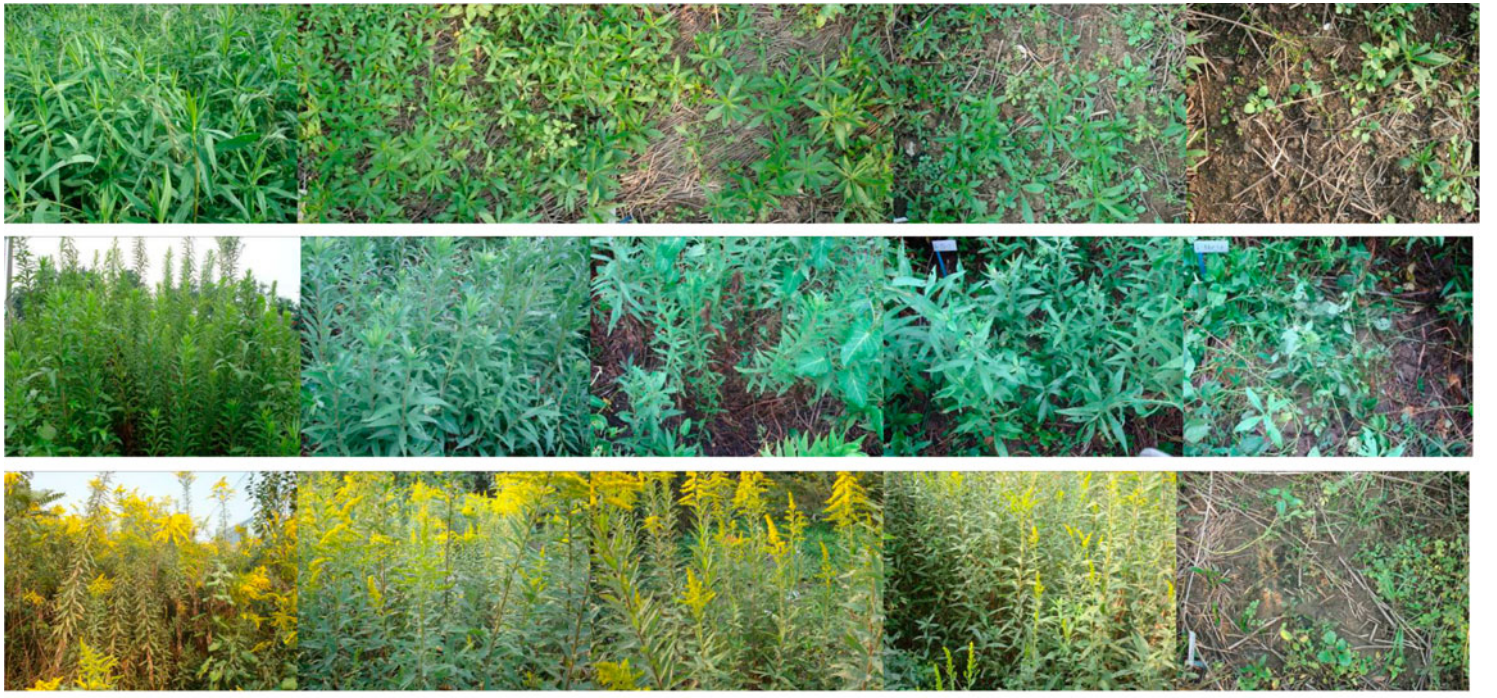

Figure 4. Visual effects of different control methods on *S. canadensis* following the mid-May trial. Images in the top row were taken two months after treatment, those in the middle row were taken four months after treatment and those in the bottom row were taken at five months after treatment where UT: untreated control; C: cutting; CSc: cutting + *S. rolfsii* SC64; CH: cutting + hoeing; CHSc: cutting + hoeing + *S. rolfsii* SC64.

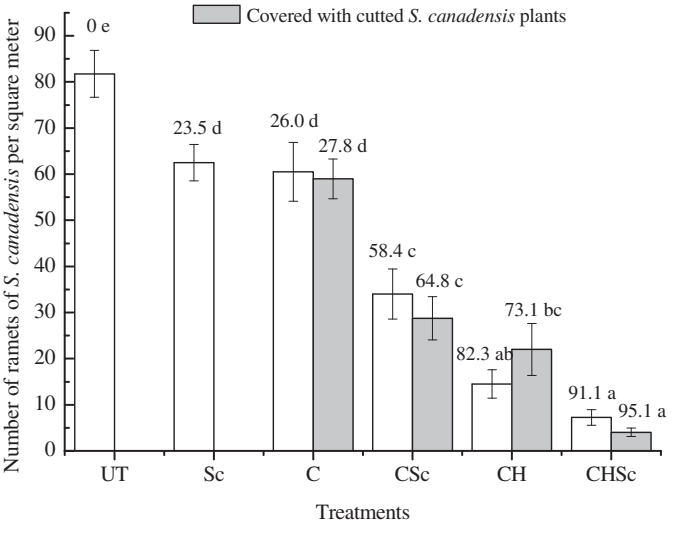

Figure 5. Mean (±SE of the five quadrats in each treatment plot) number of ramets/m^2 of *S. canadensis* following different control treatments in a waste land, where UT = untreated control; C = cutting; CSc = cutting + *S. rolfsii* SC64; CH = cutting + hoeing; CHSc = cutting + hoeing + *S. rolfsii* SC64. Shaded bars indicate treatments that were covered by cut *S. canadensis* plants, while clear bars indicate uncovered treatments. Values above the bars indicate the percentage ramet reduction in relation to the control (UT). Bars designated by different lower letters are significantly different ($P < 0.05$).

general, there was no significant effect ($P < 0.05$) of covering the plants after the different treatments.

Regeneration capability of stem sections

All stem sections that were separated from their parent plants at the 100 cm growth stage, and placed in wet soil conditions, were able to produce viable roots and develop leaves within 14 days. All sections with fibrous roots (i.e. that were taken at 0–10 cm above-ground level) developed into new ramets, while those without fibrous roots displayed over 50% regeneration rate (Figure 6). Regeneration rate was highest of sections with fibrous roots and was significantly ($P < 0.05$) higher (100%) than all other stem sections. Clearly, cut stems of *S. canadensis* should not be carelessly disposed of in the field, since even a stem section of *S. canadensis* is potentially a new source of dispersal.

Discussion

Our results showed that formulations of *S. rolfsii* SC64 caused disease symptoms and mortality in *S. canadensis* at different growth stages. However, adult plants are less susceptible to infection by *S. rolfsii*. Such age-related resistance has also been observed in other plant species, some of which became more resistant to normal virulent pathogens (Rusterucci et al., 2005). Although *S. rolfsii* was able to kill most

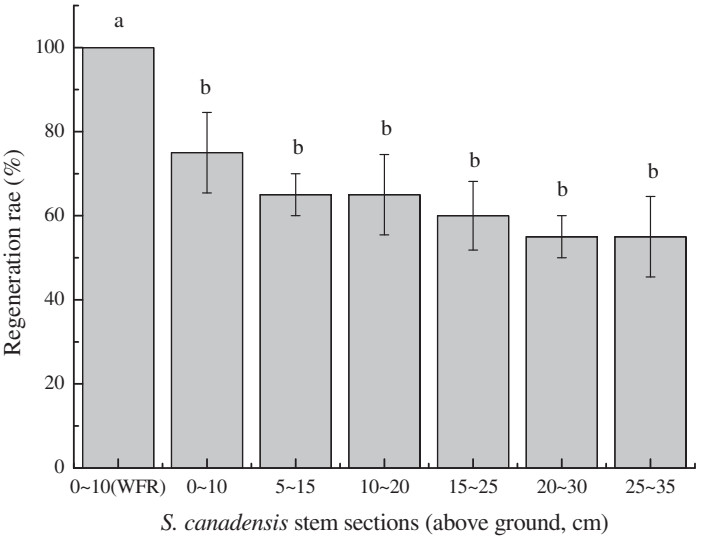

Figure 6. Regeneration rate of different *S. canadensis* stem sections above-ground (WFR represents 'with fibrous roots'). Bars designated by different lower letters are significantly different ($P < 0.05$).

above-ground material of *S. canadensis*, the underground rhizomes are not killed and can form new ramets quickly. Therefore, additional interventions are needed to control the underground rhizomes of *S. canadensis*.

The method of initial treatment and treatment timing had a substantial influence on the efficiency of control for *S. canadensis*. The removal of the plant's above-ground parts by clipping could greatly reduce its energy allocation to its below-ground structures, leading to reduced regrowth potential (Haferkamp & Karl, 1999; Orodho & Trlica, 1990). This suggests that control techniques should target the stored reserves in the roots as much as possible, and thereby reduce the resources allocated to vegetative structures, storage organs (rhizomes) and reproductive tissues. From this study, it is clear that isolated treatments involving cutting, hoeing or bioherbicide application do not sufficiently control *S. canadensis*. However, mechanical treatments damaged the plant's root system, making it more susceptible to infection by isolate SC64. Therefore, efficient control methods should be based on an appropriate combination of cutting, hoeing and treating with *S. rolfsii* SC64.

The optimum temperature range for *S. rolfsii* isolate SC64 mycelium growth was 25–35°C (Punja, 1985). Therefore, the period from mid-May to mid-October provides a suitable climate for field application in Nanjing. Vegetative growth of *S. canadensis* occurs from April to September, which provides a relatively long period for field applications. However, cutting *S. canadensis* ramets before mid-May did not provide good control, because *S. canadensis* has a high potential for recovery after being damaged in the sprouting season. Treatments during mid-July and mid-September were able to prevent flowering, which eliminated sexual reproduction for the year, and effectively inhibited vegetative growth in the same year, or recovery in the next year. From this perspective, the combination of cutting, hoeing and

inoculation with *S. rolfsii* SC64 had a positive effect on restricting *S. canadensis* in the short term (i.e. for the duration of this study).

The regeneration of stem and rhizome fragments was observed in *Fallopia japonica*, an invasive plant in UK (De Waal, 2001; Francis, Riley, & Hoggart, 2008), and rivers are common vectors for the fragments dispersal to make this weed common within many riparian areas (Dawson & Holland, 1999). However, the regeneration ability of stem sections of *S. canadensis* was not observed previously. *S. canadensis* not only reproduce from seed in its invaded area, but its ability to regenerate vegetatively from small fragments of stem and rhizome may be responsible for its prevalence in the environment, as such fragments are easily disseminated when cutting or removal is attempted.

It is practically impossible to eradicate an established invasive plant using a single control application. Usually, the uprooting of small plants can be implemented year round to control some invasive species (Sheppard, Hodge, Paynter, & Rees, 2002). Most control methods, indeed, need to be implemented several times, which leads to high costs (Genovesi, 2005; Hansen & Wilson, 2006). Based on our data, a single application of cutting, hoeing and fungal inoculation (CHSc) provided the most efficient and stable results, which may have potential for development into a regime for the control of *S. canadensis*. However, additional studies are required to evaluate the performance under field conditions, assess the risks to non-target plant species and, determine the possibility of planting crops for replacement.

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