2021: 21: 202-208

Received: 30 May 2021 | Revised: 10 October 2021 | Accepted: 11 October 2021

DOI: 10.1111/wbm.12239

TECHNICAL REPORT

Weed Biology and Management WILEY

Japanese knotweed control with winter soil injection of chemicals targeting the rhizome system

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Communicated by: Hideki Ohdan

Abstract

To achieve chemical control of aggressive creeping perennial weeds and avoid the disadvantages of conventional foliar applications, we established a winter soil-injection method using soil-active herbicides. Our previous study demonstrated the feasibility of the practical use of this method by showing that the regeneration from rhizomes or creeping root segments was inhibited by direct contact with chemicals in the soil. Then, a field trial was conducted on a population of Japanese knotweed. A stand of 148 plants growing on a railway bank was used for the experiment involving two herbicide treatments and an untreated control. In early March 2016, 1 L of chlorpropham (230 mg/L) or tebuthiuron (100 mg/L) was injected 15–25 cm deep into the base of each dead shoot clump. New shoot growth started in late April and became senescent in November. Aerial growth, determined as total shoot length and shoot number, significantly decreased throughout the season under both chemical treatments, without showing any injury symptoms (shoot dieback, leaf desiccation, or yellowing) that make unsightly views. In November, rhizome number was as low as 6 and 18% of the untreated control in the chlorpropham and tebuthiuron treatments, respectively. Sprouting and rooting abilities were reduced by treatment with tebuthiuron. Such marked reductions in rhizome number and viability, as well as the reduction in food reserves as estimated from diminished aerial growth, suggested probable substantial growth inhibition in the following season. Further improvement of the injection methodology would be attained from the consolidation of practical application data.

KEYWORDS

chlorpropham, creeping perennial weed, invasive species, soil-active herbicide, tebuthiuron

1 | INTRODUCTION

Japanese knotweed (*Fallopia japonica* [Houtt.] Ronse Decr.), a native of Asia introduced to North America and Europe in the 19th century, is a vigorous rhizomatous perennial species distributed widely across the cool-temperate to temperate zones of the world. Highly invasive, the species became a noxious weed that excluded native vegetation and reduced ecological diversity in Canada, northeastern and eastern United States, some European countries, and Oceania (Aguilera, Alpert, Dukes, & Harrington, 2010; Barney, Tharayil, DiTommaso, & Bhomik, 2006; CABI, n.d.; Gerber et al., 2008). The Invasive Species Specialist Group (ISSG/IUCN, 2004) has identified Japanese knotweed as one of the world's worst, invasive alien-species. In the UK, its exponential spread has been recognized as such a serious threat that, a person who allows it to spread into the wild may be fined or sent to prison (Knotweed Help, n.d.). In Japan, the species has existed as a native ruderal. It was utilized for folk medicine, and its sprouts were eaten by older people, but it has recently become a severe threat (Ito, 2020). Stands of knotweed often form patches on road verges, railway embankments, and riversides, interfering with the use of such infrastructure as, for example, by obstructing the view in traffic and causing biomass increase, resulting in extra costs and energy losses in vegetation management. The plants also appear to cause severe damage to trees on roadsides and in parks, because of their competitive features.

The intensive-mowing regimes to which all these knotweed habitats are permanently subjected to promotes the rapid regrowth of knotweed plants, as cutting of mature shoots overcomes apical dominance, whereby, short-term cutting results in an expanded clonal radius and an increased shoot density (Barney et al., 2006). Therefore, it is necessary to establish a reliable method to control this weed. However, Japanese knotweed is highly resistant to control efforts because of its extensive and persistent rhizome system. To date, no method has proved effective for long-term control or eradication of the species (Bashtanova, Beckett, & Flowers, 2009; CABI, n.d.), although several foliar herbicides are known to reduce its abundance (Boyed, White, & Larsen, 2017; Hagen & Dunwiddie, 2008; Rudenko & Hulting, 2010). However, foliar herbicide application to established large plants has esthetic, economic, and ecological disadvantages. Accordingly, we investigated the possibility of soil injection of soil-active herbicides to control hazardous perennial weeds developing large underground network systems. Our previous study (Ito & Ito, 2021) verified that the buds sprouting from rhizomes or creeping roots of seven tested species were effectively suppressed by one or more soil-active herbicides in direct contact in the soil. Thus, chlorpropham completely inhibited the rhizome-bud activity of Japanese knotweed. Based on this evidence, we aimed to confirm the effectiveness of soil injection under field conditions. In the present study, in addition to chlorpropham, we included tebuthiuron for testing as it is known to have high soil residual effects and is commonly used in non-crop areas to control broadleaf weeds and woody brushes (Weed Science Society of America, 1994). Our results proved that our soil-injection method contributed to the improvement of Japanese knotweed control.

2 | TESTS PRIOR TO THE FIELD INJECTION TRIAL

Precise administration of chemicals to the underground parts of the creeping perennials is essential for the successful application of a chemical soil-injection method under field conditions. Therefore, the distribution of knotweed rhizome systems and buds in soil, as well as the dispersal pattern of liquids in the soil, were preliminarily tested.

2.1 | Distribution pattern of the rhizome system

A plant selected for observation was grown from a rhizome segment for three seasons in a concrete block (90 × 90 cm, and 90 cm in depth) filled with sandy loam soil. In November, soil around the rhizome was removed carefully to a depth of 60 cm, such that the architecture of the complete system became visible, as shown in Figure 1. The plant has an underground core, from which more than a dozen aerial shoots, numerous rhizomes, and a few taproots had developed. The rhizomes extended downwards and continued to spread along the concrete walls after coming into contact with them. Many of these blocked rhizomes would likely penetrate deeper than 60 cm similar to unblocked rhizomes. Red overwintering buds, which are the source of the spring flush, were already densely formed around the basal center in autumn. At the nodes of the elongated rhizomes, both overwintering and the usual lateral buds were found. A few of these buds emerge naturally, but if the maternal shoot clump was removed, new shoots appeared densely within a 1 m radius. These findings suggest that among the creeping perennial weeds, Japanese knotweed is highly suitable for the application of soil-injection methods, because the target spots and timing are relatively easy to identify.

2.2 | Distribution pattern of the injected liquid

Although soil injection is widely used in agriculture, especially for liquid nutrient application and soil fumigation, there is little information on how far soil-injected liquids can disperse. Distances of injected liquid distribution in the soil were measured in a non-crop area (soil type: loam) with a thin cover of weed vegetation, using the same injector as applied in the field trial described later. The experiment was designed as shown in Figure 2, in which the depth of injection was determined to be 15, 20, and 25 cm below the soil surface. Filter papers 10 cm in diameter were set vertically, with the middle at the level of the injection tip. The papers were placed 30, 40, and 50 cm distant from each injection point, at each of which, 1.0 L water dyed with a cut-flower colorant (blue) was injected; 30 min later, the filter papers

FIGURE 1 A structure of the subterranean system of Japanese knotweed (left) and the aboveground feature (right), after grown three seasons in the experimental concrete block of 90 × 90 cm, and 90 cm in depth

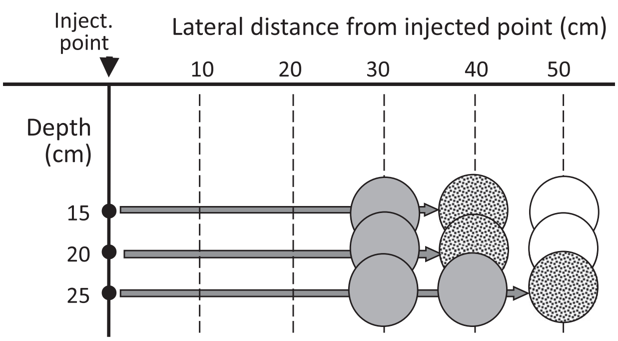

FIGURE 2 Diagram of lateral movements of colored water in soil injected at 15, 20, and 25 cm depths. Dyeing levels of filter papers (circles) placed at the different distances from injected tops were detected: gray, dotted, and open circles indicate fully, partially, and not dyed, respectively

were withdrawn from the soil, and the amount of dye was visually examined. At all three injection depths, papers located at 30 cm were fully dyed, and the injected liquid was found to reach further laterally as the injection depth increased, reaching 50 cm when injected at a depth of 25 cm. These results indicated that one injection 15–25 cm deep may cover the area within a radius of 30 cm.

3 | FIELD TRIAL OF SOIL INJECTION

3.1 | Site and methods

The site for this trial was part of a local railway bank (gradient: 30°, 8 m wide) located in Miyazu City (35°58′N, 135°19′E), Kyoto Prefecture, Japan. A distance of 24 m along the bank, where a sufficient number of established knotweed plants were available, was selected. The site (soil type: loam, pH: 5.3) was previously disturbed by covering with a weed-proof transparent sheet and planting *Phlox subulate* (a cover plant) seedlings in holes spaced 50 m apart. However, 3 years after when this trial started, most of the *Phlox* plants had been replaced by weeds, mainly by knotweed. Thus, a suitable area for our trial appeared accidentally.

The plots (1.5 × 7 m) for chlorpropham, tebuthiuron, and the untreated control were arranged 2 m apart from each other within two rows on the slope. Treatments were conducted in February 2016 using the equipment shown in Figure 3. Chlorpropham and tebuthiuron were dissolved in 50 L of water in a tank at a concentration of 230 mg a.i. /L and 100 mg a.i./L, respectively. The solutions were forced out at 1.5 MPa to the injector through a 100 m hose, and 1 L per sheet hole was injected into each treatment plot, whether knotweed clumps were present or not. One injection lasted approximately 3 s under 0.2 MPa. Injection depths were 15–20 cm for chlorpropham and 20–25 cm for tebuthiuron. As all holes were spaced 50 cm apart, the chemicals were considered to be fully dispersed laterally, judging from the preliminary test.

To estimate the effects of soil injection of chlorpropham and tebuthiuron on growth, plant height was classified by 50 cm intervals, and the number of all treated and untreated plants belonging to each class was counted in May and August: 33 plants for chlorpropham, 55 for tebuthiuron, and 60 for the control. At the same time, the presence of injury symptoms (shoot dieback,

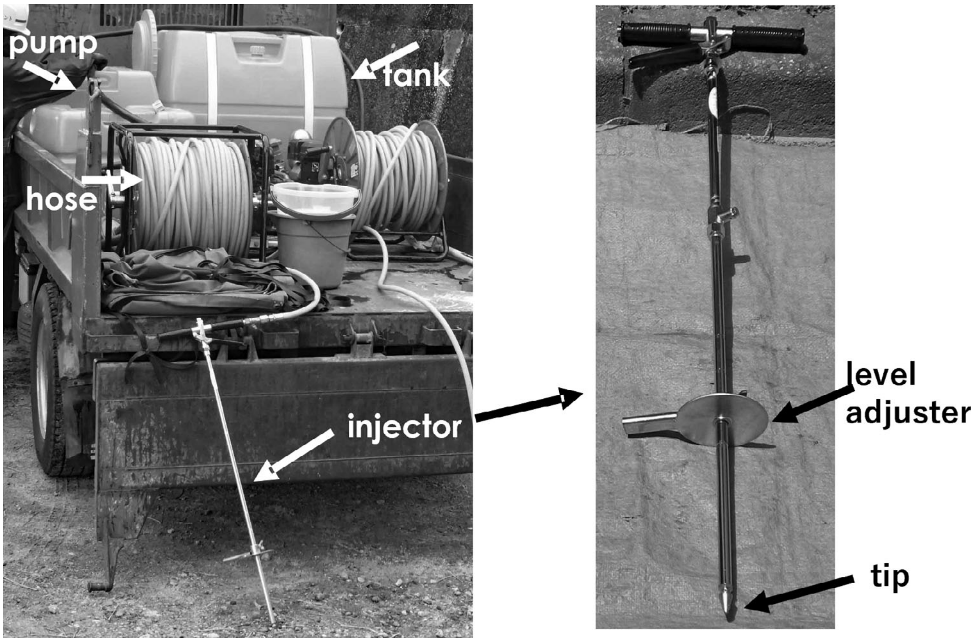

FIGURE 3 Equipment used for the soil injection treatment, consisting of a tank, a pump, and an injector with a connected hose that transports liquids to the injection point

leaf desiccation, or yellowing) was checked visually. Ten relatively large plants of similar size (selected based on the feature and number of died-back shoots in a clump) were fixed in each treatment for continuous determination of shoot growth. The number and total length/shoot were measured in May, August, and November. Shoot diameter 10 cm above the ground was also measured. In November, to estimate the amount of rhizome growth, all rhizomes in the soil to an approximate radius of 25 cm and at 25 cm death around a plant clump were excavated for 3 of the 10 plants and weighed for fresh weight. At the start of this trial, we confirmed, by digging a part of the area, that features of rhizome distribution were basically similar to those recognized in the preliminary test (Figure 1), lateral rhizomes from the clump base intertwined between plants, and some of them penetrating deeper than 60 cm. Thus, we knew that harvest was not complete, but excavating all the rhizomes without causing excessive damage to the bank was very difficult. After weighing, the rhizomes were used to test bud viability. First, they were cut into segments (6–10 cm) with one or more nodes and allowed to regrow for 3 weeks on wet paper towels at room temperature (20–25°C). The number of sprouted and rooted segments was counted. Fifteen segments were used for each treatment.

Data on shoot length at three measuring times and shoot diameter in November were analyzed using one-way ANOVA, and differences in treatment means were assessed using the Tukey–Kramer test. Percent differences in plant height distribution and number of sprouted or rooted rhizome segments were assessed using the Chi-squared test.

3.2 | Results

Japanese knotweed shoots at the site emerged from the base of the clumps around mid-April, grew vigorously to their maximum in August, and began to senesce in November. Aerial growth as estimated by the distribution of plant height was reduced significantly under treatment with chlorpropham throughout the growing season, compared with the untreated control, whereas, growth reduction occurred late in the season under treatment with tebuthiuron (Figure 4). Despite growth reduction, no injury symptoms such as dieback or leaf desiccation, or yellowing were observed either in chlorpropham- or in tebuthiuron-treated plants. The number and total length of shoots, essential components of aerial growth, represented by the means for 10 fixed plants, changed almost in parallel to the aerial growth expressed in terms of plant height (Table 1). The values obtained in November showed that senescence tended to occur earlier in herbicide-treated plants than in untreated controls. Furthermore, shoots were thinner in the chlorpropham treatment, where growth was inhibited at an early stage.

The growth of rhizomes in herbicide-treated plants was extremely reduced: relative to the control (Table 2), their fresh weights measured in November were 5.5 and 18.2% in chlorpropham and tebuthiuron, respectively.

Rhizome bud activities as evaluated by sprouting and rooting from the segments were significantly inhibited by the tebuthiuron treatment (Table 2) where most initiated roots stopped growth at the epidermis, even 10 months after injection (Figure 5). Interestingly, callus was formed at the basal end of the segments despite that the main

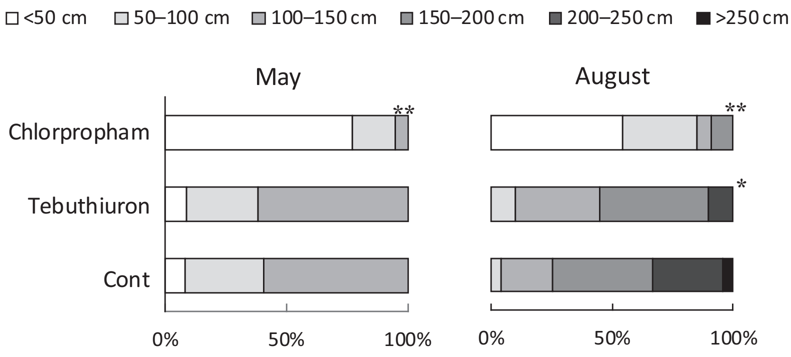

FIGURE 4 Plant height distribution of Japanese knotweed in herbicide-injected and untreated areas. Thirty-three plants for chlorpropham, 55 for tebuthiuron, and 60 for the untreated control were measured. * and ** indicate significant differences relative to the control treatment in May and August, according to the Chi-squared test at $p = .05$ and $p = .01$, respectively

TABLE 1 Japanese knotweed shoot responses to soil injected chlorpropham and tebuthiuron

	Total shoot length/plant (cm)			No. of shoot/plant			Shoot base diameter (mm)	Phytotoxic
Treatment	**5/18**	**8/5**	**11/18**	**5/18**	**8/5**	**11/18**	**11/18**	**symptom**
Chlorpropham	52.2b	171.3b	66.0b	1.6b	2.9a	1.0b	6.3b	None
Tebuthiuron	189.0a	259.8ab	121.0b	3.8a	3.9a	1.5b	8.3a	None
Control	203.0a	416.9a	341.5a	3.5a	4.1a	3.3a	10.8a	None

Note: Means within a column followed by the same letter are not significantly different according to Tukey's test at $p = .05$.

TABLE 2 Fresh weight of rhizomes harvested in November and the ability of sprouting and rooting of rhizome segments

Treatment	Flesh weight (g/plant)	Sprouted segments (%)	Rooted segments (%)
Chlorpropham	85.0a	83.3	87.3
Tebuthiuron	282.2a	40.0**	33.3**
Control	1,546.0b	93.3	80.0

Note: Means in the fresh weight followed by the same letter are not significantly different according to Tukey's test at $p = .05$. Sprouting and rooting were tested for 15 segments, and ** indicates significant differences relative to the untreated control, according to the Chi-squared test at $p = .01$.

mechanism of action of tebuthiuron is to inhibit photosynthesis.

The above results confirmed that both chlorpropham and tebuthiuron injected into the soil in winter, when rhizome buds were quiescent, inhibited knotweed growth in the following season, but the timing of their effects was different: the effect of chlorpropham appeared early and continued throughout the season, whereas that of tebuthiuron appeared late in the season.

4 | DISCUSSION

Foliar application of phloem-mobile herbicides is generally recognized as an effective means of controlling creeping perennial weeds by suppressing growth, development, and the function of underground networks of rhizomes or creeping roots. Herbicides, such as glyphosate, synthetic auxins, asulam, and aryloxyphenoxipropionate herbicides (for grasses) have been reported for their active downward translocation and suppression of bud viability (Carlson & Donald, 1988; Harker & Dekker, 1988; Ito & Asai, 1995; Ito, 2000; Tardif & Leroux, 1990; Veerasekaran, Kirkwood, & Fletcher, 1977). Rhizome system suppression was unexceptionally important for Japanese knotweed control. Several trials have revealed that chemical methods using phloem-mobile herbicides are superior to any other mechanical, physical, or biological methods, although none of them has succeeded in eradicating this weed (Barney et al., 2006; Bashtanova et al., 2009; Boyed et al., 2017; CABI, n.d.; Hagen & Dunwiddie, 2008). Moreover, no previous study has reported on the responses of rhizome systems or rhizome buds, although they are the real targets of control.

The aggressiveness of Japanese knotweed can be explained by its ability to expand its territory through rhizome networks, from which shoots regenerate, and its additional ability to colonize by fragmented rhizomes. In fact, this species is known to spread asexually rather than through seeds (Barney et al., 2006). Even a small piece of fragmented rhizome can regenerate into a new plant (CABI, n.d.); indeed, 70% of new plants were found to originate from rhizome fragments in a flood-dispersed knotweed population (Colleran & Goodall, 2014). In the present study, both chlorpropham and tebuthiuron

FIGURE 5 Abnormal rooting response of the rhizome segments to tebuthiuron in November, 9 months after soil-injection treatment. Roots no longer developed out of the epidermis (A: white dots) but the callus was well formed at the cut end (B). (C) Normal growth in the untreated control

significantly reduced knotweed growth. The most remarkable effect was the apparent inhibition of the amount and viability of rhizomes, which reduced growth in the following season. Knotweed plant growth, particularly the spring flush and subsequent early growth, largely depends on the extent of the rhizome systems and the amount of food reserves they can store. Both chemicals tested herein suppressed the knotweed rhizomes significantly. The number of rhizomes for plant expansion and regeneration decreased, and the downward translocation occurring mainly in autumn (Kashino & Ito, 1996; Price, Gamble, Williams, & Marshall, 2002) was likely scarce, slow, or both. Moreover, rhizome bud viability was weakened. Overall, these results suggest that two successive injections might lead to the successful eradication of this weed.

Our challenge was to establish an effective winter soil-chemical injection that can reduce the esthetic, economic, and ecological disadvantages that commonly arise as a result of foliar applications. From this viewpoint, it is noticeable that the inhibited knotweed plants in our trial did not exhibit any signs of injury, such as dieback, leaf desiccation, or yellowing, which cause unsightly scenes when foliar chemicals are applied. Further information is required to improve the injection technology and develop more effective chemicals. The vertical dispersion of the injected liquid in the soil was not clarified in the preliminary test. Slow responses in tebuthiuron-treated plants might have resulted from deeper injections than necessary. Our study was conducted at a site with loam soil, where the size of the knotweed plants was relatively uniform. However, the growth conditions and plant sizes of knotweed vary among habitats, and the injection methodology should be adjusted on a case-by-case basis. Case studies based on the analysis of data from multiple practical uses would facilitate the improvement of soil-chemical injection as a means of knotweed control.

ACKNOWLEDGMENTS

The authors would like to thank Toshiharu Miyazaki, Makoto Ikemura, and Mai Konishi for their practical assistance with the field trials. We also thank Ascent Co. Ltd. for providing financial support.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Ito, M., & Ito, K. (2021). Japanese knotweed control with winter soil injection of chemicals targeting the rhizome system. *Weed Biology and Management*, 1–7. https://doi.org/10.1111/wbm.12239