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ORIGINAL ARTICLE

Time to kill the beast – Importance of taxa, concentration and timing during application of glyphosate to knotweeds

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Abstract

Knotweeds (*Fallopia* spp., syn. *Reynoutria* spp.) are among the most invasive plants globally, mainly due to their ability to regenerate from rhizomes and their extremely high biomass production. Spraying with glyphosate is a common control method, yet little is known about its effectiveness on underground rhizomes. In addition, there are concerns about the negative environmental impact of glyphosate. Therefore, it is essential to use appropriate dosages and application times to avoid overuse. Based on a pot trial and field experiments, we assessed the effectiveness of glyphosate concentration, application time, and influence of glyphosate on rhizomes from different soil depths to determine their effect on the aboveground and belowground parts of knotweed plants of different taxa. The study demonstrates that sampling rhizomes is a more consistently accurate indicator of knotweed regeneration rate than sampling shoots. Regeneration of shoots and rhizomes was affected differently by glyphosate spraying depending on the application time. The effect on rhizomes was much greater with early season spraying than late season spraying, which primarily reduced shoot biomass. However, no differences were found between rhizome vitality at different soil depths. *F. sachalinensis* was sufficiently controlled by early season foliar spray with 5% glyphosate (3.65 kg a.i. ha^{-1}) in contrast to *F. japonica* and *F.* ×*bohemica*. For rapid and targeted control, early season foliar spray with 8% glyphosate (5.85 kg a.i. ha^{-1}) is needed and, in the case of the hybrid, for a minimum of two consecutive seasons.

KEYWORDS
alien species, chemical control, eradication, *Fallopia* spp., herbicide, invasive species, *Reynoutria* spp., weed management

1 | INTRODUCTION

The spread of invasive alien weeds is one of the main threats to the environment today (Neve et al., 2018). Knotweeds are among the worst European invaders (Netwig et al. 2018) and cause serious problems worldwide (e.g. Clements et al., 2016). Knotweeds have spread uncontrollably despite nature conservation management efforts. They are invaders of riparian and anthropogenic habitats, where they form perennial stands that can outcompete other species completely, resulting in loss of biodiversity and limiting many ecosystem services (Abgrall et al., 2018; Murrell et al., 2011). Localities in urban habitats, parks, and gardens may be particularly hard-hit by large monoculture knotweed stands (Chmura et al., 2013; Sołtysiak and Brej, 2014), and can represent hotspots for future spread (Vojík et al., 2020). Recently, an increasing spread has also been registered in agricultural fields with potato or maize (Skinner et al., 2012). There is evidence of such a risk for Central Europe as well (Figure 1).

Herbicides are routinely used as the most effective control method for invasive plants (Kabat et al., 2006; Majd et al., 2019; Rudenko and Hulting, 2010). However, the application does not always lead to the total eradication of invasive stands (Rinella et al., 2009). Incomplete eradication can lead to new spread (Blossey,

FIGURE 1 Spread of invasive *F.* ×*bohemica* into a maize field

1999). Optimal control methods can be developed by identifying the appropriate herbicide dose and optimal plant response to spraying at different developmental stages (Benbrook, 2016; van Bruggen et al., 2018). The dose of herbicide that ensures optimal control effectiveness is related to environmental conditions (temperature, soil moisture, etc.); specific plant traits such as species, plant stand size, or developmental stage; and the application time. The growth season sees an increase in plant biomass as well as the changes in physiological response to herbicides. For example, the biomass of knotweed stands is considerably higher in autumn than in spring or early summer, and different taxa exhibit different levels of sensitivity to herbicide control (e.g. Jones et al., 2018) due to physiological characteristics (Bashtanova et al., 2009). This increased biomass will require a greater amount of herbicide for complete control, which in turn increases the risk of environmental contamination.

The most broad-spectrum of the currently approved herbicides used for weeds control, particularly for knotweeds, is glyphosate (N-(phosphonomethyl)-glycine-isopropylamine (IPA) salt) (Jones et al., 2018), commonly sold in the commercial formulation, Roundup – Active, Rapid, etc. (Bayer Crop Science). Instructions for its application generally recommend the lowest applicable dose in natural ecosystems to minimise the risk of any potential impact on the environment or human health; however, studies considering taxa-specific response, physiology, and appropriate glyphosate doses for knotweed are missing.

Various knotweed control methods have been attempted (Dommanget et al., 2013, 2019; Kabat et al., 2006). Most of them combine mechanical control with the use of herbicides (Bashtanova et al., 2009; Jones et al., 2018; Kabat et al., 2006). However, mechanical cutting of aboveground biomass, such as mowing, only results in weakening of the stands and not complete eradication (e.g. Scott and Marrs, 1984). Knotweeds, like many perennials, can store carbohydrate in their underground rhizomatic systems. Such resource allocation creates an enormous propagule source underground combined with extremely high rhizome bud regeneration ability. For this reason, stand-alone digging up of whole stands is most effective, but technically difficult and only applicable to smaller knotweed stands (Barták et al., 2010). Digging and cutting the rhizomes into small segments leads to rapid rhizome segment regeneration. Followed-up with spraying with glyphosate appears to be most effective but technically difficult method (Bímová et al., 2003). In Europe, late season spraying is widely considered as one of the most effective control methods. This involves spraying of glyphosate (5%–10% liquid concentration) in autumn (Barták et al., 2010; Kabat et al., 2006). During the late vegetation season, assimilates are stored in the rhizomes, and it is believed that this process helps transport the glyphosate to rhizomes and kill them. Within this process, the plants must not be ripped, dug, or mechanically damaged. The effectiveness of late season spraying in reducing aboveground biomass is high and has been described (Jones et al., 2018; Kabat et al., 2006). Nevertheless, little or no information is available on the effect of this method on rhizomes in clonal plants and subsequent regeneration from rhizome buds (Bímová et al., 2003; Pyšek et al., 2003), despite the importance of the knowledge of rhizome regeneration rate in evaluating control efficiency over the following seasons. Other common methods include cutting and follow-up spraying with glyphosate or spraying in the mid-summer vegetation season (Barták et al., 2010). In such cases, the efficiency is usually evaluated by counting newly regenerated shoots, often neglecting the underground parts (Jones et al., 2018). As mentioned above, the rhizome systems of the taxa are complex in architecture and provide long persistence; they are able to penetrate almost all types of substrates and can be found at a distance of 20 m from a maternal stand (Beerling et al., 1994). Plants can regenerate from tiny fragments of rhizomes (1 cm, 0.7 g) containing at least one node with a bud (Adachi, 1996; Brock and Wade, 1992). Although there is a large body of knowledge, no studies have focused on the response of underground plant parts to particular control methods. Despite proper application, glyphosate is not always distributed to deep-seated rhizomes and thus could lead to plant regrowth.

Studies on knotweed control also suffer from a lack of taxa comparison. Studies have mainly focused on the control of *F. japonica* Houtt. var. *japonica* (Japanese knotweed; Kabat et al., 2006), in spite of apparent differences in the biology of particular taxa. As such, related taxa could respond differently to control treatments, as suggested by Bímová et al. (2003). Previous studies on the regeneration ability of *F. japonica* var. *japonica* (herein *F. japonica*) and *F. sachalinensis* (F. Schmidt) Nakai (giant knotweed), and their hybrid *F.* ×*bohemica* Chrtek et Chrtková (Bohemian knotweed) (Bímová et al., 2003; Kabat et al., 2006; Ringselle et al., 2021) and their competitive fitness (e.g. Parepa et al., 2014) indicate apparent differences, with rhizomes of *F. japonica* and *F.* ×*bohemica* having a recovery capacity nearly twice that of the aboveground stems. Furthermore, while *F.* ×*bohemica* is considered the most regenerative taxon of the genus, *F. sachalinensis* tends to regenerate better from stems (Bímová et al., 2003). Hence, the taxa-focused method should be developed to optimise control effectiveness.

To fill this gap, this study used a combination of pot trial and field experiments to find the optimal knotweed control method for particular taxa. The objectives of the study were to consider shoot emergence and rhizome regeneration ability after summer (early season) and autumn (late season) application of the foliar spray glyphosate.

We tested commonly used dose of glyphosate (5%, 3.65 kg a.i. ha^{-1}) and the highest dose (8%, 5.85 kg a.i. ha^{-1}) suggested by the producer of Roundup®Active for the most resistant weeds. The study also compared effects of glyphosate treatments on rhizomes at different depths, as a possible important factor influencing the control effectiveness.

2 | MATERIAL AND METHODS

Three taxa of the genus *Fallopia* were selected (i.e., *F. japonica*, *F. ×bohemica*, *F. sachalinensis*) and subjected to various glyphosate treatments. Roundup®Active, containing the active ingredient glyphosate ($C_3H_8NO_5P$) 170 g L^{-1} SL (IPA salt), was applied by foliage spraying at 5% (3.65 kg a.i. ha^{-1}) and 8% (5.85 kg a.i. ha^{-1}) concentration dose during early season and late season, when the plants were at different developmental stages to determine the response of above/underground parts for comparing the efficacy of different doses of glyphosate.

The average spray volume, doses, and mean amount of aboveground biomass are shown in Table 1. No adjuvants were used.

This study was based on one ex situ pot trial and two in situ field experiments. All experimental plots and sample collection plots were located in Central Bohemia in the Czech Republic (see Figure S1).

2.1 | Garden pot trial – comparison of treatment times, glyphosate concentration, and taxa

Experimental plants were obtained from rhizomes that had been transported to a greenhouse, cleaned, and put to regenerate in water until new shoots formed. The regenerated rhizomes were placed in separate pots filled with a mixture of perlite and sand (1:1). All rhizomes were 25 cm long, consisting of approximately ten nodes with rhizome buds and one newly regenerated shoot. The experiment was conducted between May and October 2015, during which the plants were fertilised three times and watered regularly. Replication comprised 15 plants of each taxon treated with different concentrations of glyphosate (5%, 8%), and 15 plants left unsprayed as a control. Spraying was carried out in early season and late season. Two sprayings using a classic knapsack sprayer (Titan 16 VITON, Marolex) were conducted for each of these periods. Early season spraying was performed in the last week of May and 3 weeks later. Late season spraying was performed in the first week of September and 3 weeks later. After 4 weeks (i.e., in July for early season spraying and October for late season spraying), the rhizomes were cut into segments (each consisting of one node with two adjacent internodes), washed, and moved to containers with distilled water, where regeneration was observed under greenhouse conditions. Rhizome bud regeneration was recorded every second day for 1 month. The segments were considered to be regenerating if they produced new shoots from rhizome buds. The number of newly regenerated shoots was counted for each segment and used as a response variable for data analysis.

2.2 | Field experiments – comparison of treatment times, glyphosate concentration, taxa, and the effects of glyphosate at different rhizome depths

The first field experiment was conducted from August 2015 to October 2017. Glyphosate was applied at two concentrations (8%, 5%) to experimental treatment plots (9 m^2 on average) of each taxon at separate localities (i.e., three repeats, different localities for each taxon). A plot of similar size, which served as the control plot for each taxon, received no glyphosate spray. The glyphosate was applied to the whole knotweed stand (i.e., spraying aboveground biomass) at each locality using a knapsack sprayer, with the initial spraying carried out in the first week of September and the second spraying three weeks later. Data were collected using a 4 m^2 plot in the centre of each test plot four weeks after the second spraying. In each plot, the number of partial clumps and number of shoots before spraying were counted (and later used as a covariate), and the number of newly regenerated shoots after spraying treatment was counted and used as a response variable in the data analysis. The plots were again examined over the following two seasons when newly emerged shoots were subjected to the same glyphosate concentrations (spot spraying).

The second field experiment was conducted between May and October 2018. Based on previous results, glyphosate was only applied at 8% concentration. Experimental plot organisation followed the methodology of the first field experiment (i.e., 9 m^2, three repeats,

TABLE 1 Details of spray volume, application dose (kg a.i. ha^{-1}), and size of aboveground biomass by treatment group. Taxa: FB – *F. ×bohemica*, FJ – *F. japonica*, FS – *F. sachalinensis*

Application timing	Taxa	Spray volume (litres ha^{-1})	Mean of application dose 5% (kg a.i. ha^{-1})	Mean of application dose 8% (kg a.i. ha^{-1})	Aboveground biomass (kg. ha^{-1})
Early season	FB	500	3.1	5.0	4394.0
	FJ	375			3976.5
	FS	225			2955.3
Late season	FB	625	4.2	6.7	8288.0
	FJ	550			7841.5
	FS	300			5479.6

different localities for each taxon), while the glyphosate season application followed the methodology of garden pot trial (i.e., two treatment times). Four weeks after the second spraying, new shoots were counted in both treatment plots, and rhizomes were dug from different depths (10–40 cm) from the inner 1 m^2 of each experimental plot. Regeneration of harvested rhizomes followed the same method as in the garden pot trial (i.e., cut into segments and rhizome bud regeneration recorded). The number of regenerated buds was counted for each segment and used as a response variable for further data analysis.

2.3 | Data analysis

Several main factor ANOVA (Analysis of variance) models were employed to detect differences in the percentage of regenerated rhizomes (arcsine transformed) with taxa, concentration of glyphosate, season, and depth of rhizome layer as predictors. GLM (generalised linear models) were employed to analyse the differences in the number of newly regenerated shoots with taxa, glyphosate concentration, and season as predictors. The initial number of partial clumps and shoots were used as covariates in the model. The minimal appropriate model was obtained using posterior contrasts (Quinn and Keough, 2002) and AIC criterion (Akaike, 1998). In addition, RM (Repeated Measure) ANOVA model was applied to interpret the between-year decrease of shoots. In the RM ANOVA model, the response variable was log-transformed ($Y' = 1 + Y$). Year (RM factor), concentration, and taxa were used as predictors. Tukey's HSD tests followed the ANOVA models.

All statistical tests and output graphics were performed using R (R Development Core Team, 2019) and Statistica 13 (TIBCO, 2017) statistical software with respect to current statistical issues (Onofri et al., 2010). Differences were considered significant at $p \le 0.05$.

3 | RESULTS

While spraying with glyphosate significantly reduced both shoot occurrence (GLM, $z = -6.93$, df = 23, $p < 0.0001$) and rhizome regeneration (ANOVA, $F_{2,264} = 677.76$, $p < 0.0001$) regardless of the concentration applied, there were significant differences in the number of new shoots produced (GLM, $z = -2.73$, df = 8, $p = 0.01$) and regeneration rate of rhizomes (ANOVA, $F_{2,264} = 677.76$, $p < 0.0001$, Tukey HSD test 5% vs. 8% <0.0001).

3.1 | Tested treatments and shoot occurrence

The number of shoots per plot decreased significantly faster after spraying with 8% concentration ($F_{4,36} = 31.33$, $p < 0.0001$). After spraying with 5% concentration, the number of newly emerged *F. ×bohemica* shoots varied from 3 to 5 per plot, in *F. japonica* from 1 to 3 shoots, and in *F. sachalinensis* from 0 to 2 shoots. In contrast, no significant difference in the number of new shoots was observed between taxa after spraying with 8% concentration (GLM, $z = 0.000$, df = 8, $p = 1.00$) and the overall number of new shoots decreased almost to zero (mean number of shoots varied from 0 to 0.33 per sample plot, Figure 2).

There was no significant interaction between the three years of study for particular treatment and taxa (RM ANOVA, $F_{8,36} = 1.22$, $p = 0.30$). If single factors were tested, there was a statistically significant decrease in shoot numbers in the second and third years in all treatments except control (RM ANOVA, $F_{2,36} = 38.68$, $p < 0.001$). There were no new shoots in the final year in plots sprayed with both concentrations in *F. sachalinensis* and 8% concentration in *F. japonica* (Figure S2, Table S1).

After spraying with 8% concentration, no differences were found in the number of newly regenerated shoots between seasons (GLM, $z = 1.27$, df = 17, $p = 0.21$) and particular taxa (GLM, $z = 0.28$, df = 17, $p = 0.80$) (Figure 3; Table S2).

3.2 | Tested treatments and rhizome regeneration

There was a clear taxa-specific pattern in rhizome reaction to glyphosate concentration applied ($F_{2,264} = 8.20$, $p < 0.0001$), *F. sachalinensis* rhizomes regenerated equally after spraying with both concentration (mean c5% = 10.22%, c8% = 7.46%; c = concentration) other taxa were more reduced by 8% glyphosate (*F. japonica* – mean c5% = 18.69%, c8% = 9.47%, *F. ×bohemica* – mean c5% = 19.68%, c8% = 11.8%) (Figure 4).

The application season significantly affected rhizome regeneration rate (ANOVA, $F_{1,264} = 28.59$, $p < 0.0001$), with a lower regeneration rate in early season spraying compared with late season spraying. While *F. ×bohemica* and *F. japonica* rhizome regeneration rates were significantly higher (mean early: 13.2% and 9.72%; mean late: 26.15% and 27.65%) than *F. sachalinensis* (mean early: 6.57%, mean late: 13.88%) after spraying with 5% glyphosate concentration (Tukey HSD, $p = 0.01$), there was only a small difference in the rhizome regeneration rate between all taxa using 8% glyphosate concentration (for specific results, see Tables S3 and S4).

The effect of different depths of rhizomes in the soil on regeneration rate was not statistically significant ($F_{3,71} = 0.662$, $p = 0.58$) (for details see Table S5).

4 | DISCUSSION

4.1 | Glyphosate concentration, shoot occurrence and rhizome regeneration

The results indicate that taxa respond differently to glyphosate concentrations treatments. In the 8% glyphosate concentration, there was little difference between taxa because the treatment strongly suppressed the growth of both shoots and rhizomes in all taxa (albeit it could not stop mainly the hybrid growth completely). However, in the 5% treatment, there was a clear difference between taxa with the

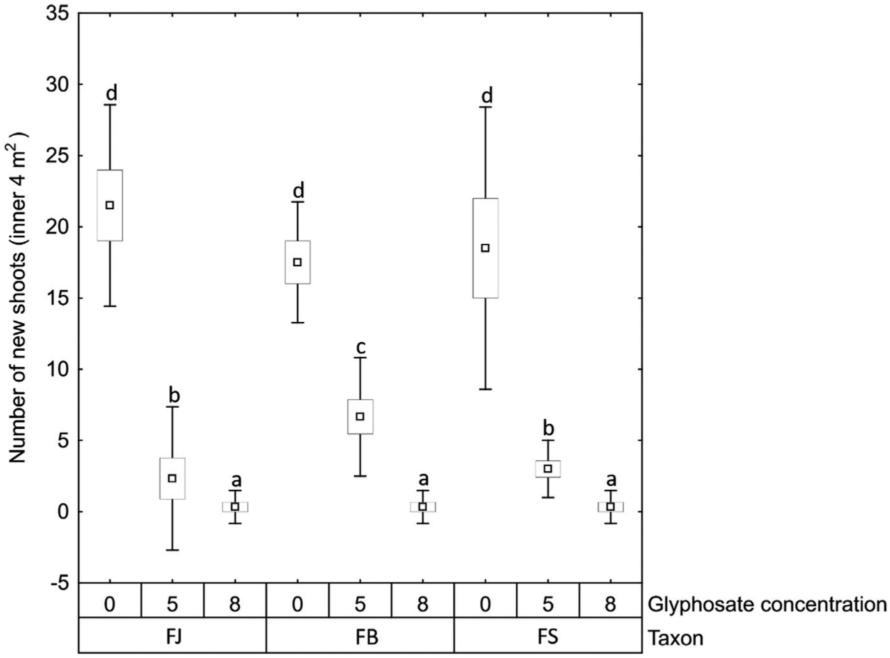

FIGURE 2 Influence of different treatments on the newly regenerated shoots (field experiment) in inner 4 m^2 after one season's spraying; glyphosate concentration 5 = 5%, 8 = 8%, 0 = without treatment; taxon: FJ = *F. japonica*, FB = *F.* ×*bohemica*, FS = *F. sachalinensis*. Squares = mean, boxes = mean ± SE, whiskers = mean ± 2SD, boxes sharing a letter do not differ (Tukey-adjusted comparisons; $p < 0.05$)

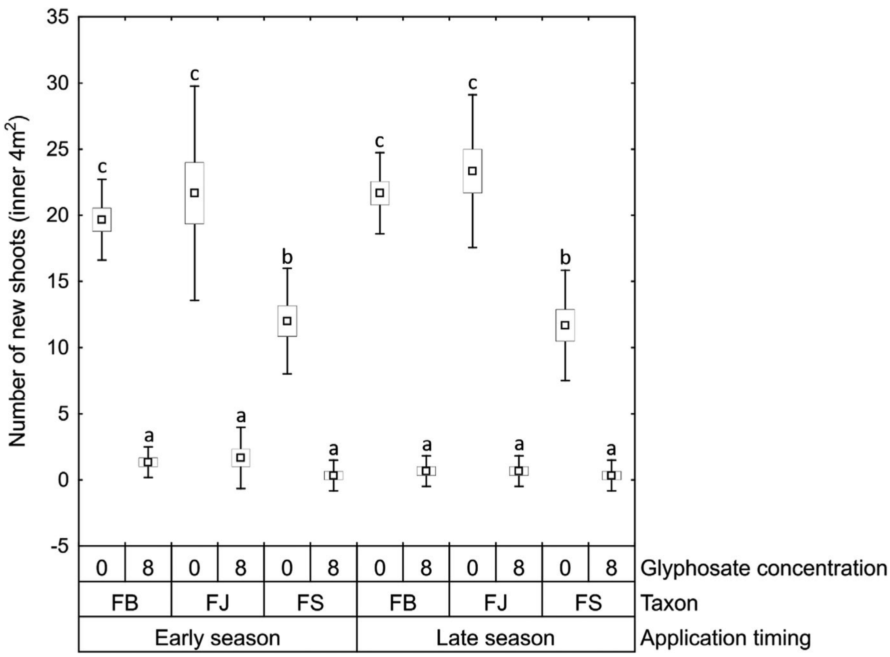

FIGURE 3 Influence of different treatments on the newly regenerated shoots (field experiment) in inner 4 m^2; glyphosate concentration 8 = 8%, 0 = without treatment; taxon: FJ = *F. japonica*, FB = *F.* ×*bohemica*, FS = *F. sachalinensis*. Squares = mean, boxes = mean ± SE, whiskers = mean ± 2SD, boxes sharing a letter do not differ (Tukey-adjusted comparisons; $p < 0.05$)

hybrid surviving far better (producing 6.67 shoots in overage) than the parent species *F. japonica* (2.33) and *F. sachalinensis* (3.0). Consequently, the 5% concentration is not effective enough for the hybrid.

Similar results were obtained for the regeneration rate of rhizomes; the rhizome regeneration results showed that even an 8% glyphosate concentration could not stop knotweed growth completely, although it reduced growth significantly.

4.2 | Glyphosate and rhizome system

Numerous authors have pointed out the complexity and high biomass of the knotweed rhizome system and the problems associated with its eradication (Bashtanova et al., 2009; Bímová et al., 2001, 2003). Brock (1995) reported that rhizome biomass can reach up to 1500 g m^{-2}. As such, systemic herbicides do not affect all parts of the rhizome system. The non-affected parts can regenerate and thus prevent complete eradication of the knotweed. This could be a significant factor influencing the success of control. Therefore, we focused on the glyphosate efficacy on rhizomes excavated from different depths of soil. We assumed that the deep-laid rhizomes would be affected less by glyphosate spraying than these growing close to stems. Surprisingly, no difference was found in influencing the regeneration of rhizomes at different depths. The probable reason is the very complex architecture of the rhizome system, with rhizomes growing up to 7 m in different directions. Even within a knotweed

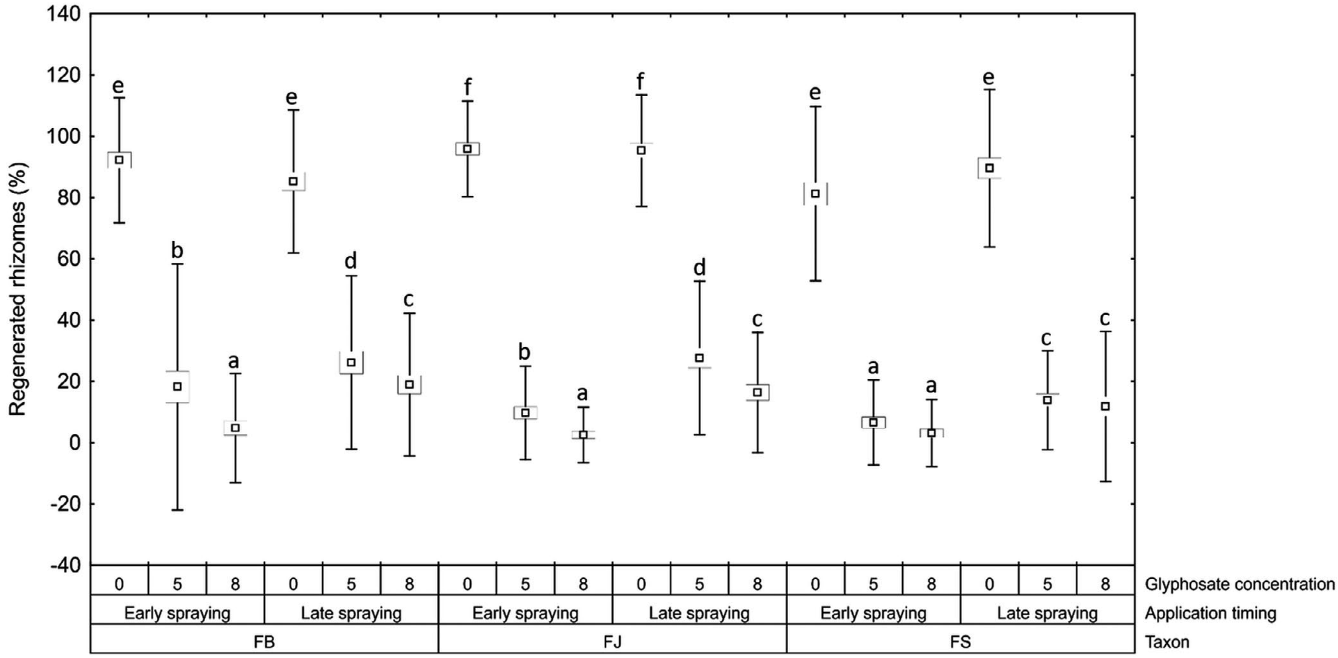

FIGURE 4 The percentage of regenerated rhizomes (garden pot trial) among *F.* ×*bohemica* (FB), *F. sachalinensis* (FS) and *F. japonica* (FJ) based on different application timing and treatment (glyphosate concentration 5 = 5%, 8 = 8%, 0 = without treatment). Squares = mean, boxes = mean ± SE, whiskers = mean ± 2SD, boxes sharing a letter do not differ (Tukey-adjusted comparisons; $p < 0.05$)

stand, rhizomes can be at different distances from the aboveground stems and, therefore, far from the site of herbicide application. Individual taxa vary greatly in the structure of their rhizome system (Berchová, unpublished data); this may be the reason for the difficulty in getting rid of the hybrid *F.* ×*bohemica*, which has relatively strong (like *F. sachalinensis*) and, simultaneously, long, deep growing, and branched rhizomes like *F. japonica*.

4.3 | The timing of spraying

Jones et al. (2018) described phenological changes in *F. japonica* growth, resource allocation, and rhizome source-sink strength during the growing season. Based on the study of phenological processes and extensive field-based assessment of control treatments, the authors suggest using biannual summer and autumn foliage spraying with glyphosate or autumn shoot injection. Both methods are based on the flow of resources in autumn, reaching a maximum in the rhizome-shoot direction. Rhizome bud activity is then triggered and exhausted. The results of our two-season experiment show that knotweed rhizome regeneration was significantly reduced after early season spraying, whereas late season spraying reduced the aboveground biomass almost completely, but rhizome bud regeneration was less affected. This was seen also in the pot trial under standardised conditions (see Tables S3 and S4). In the field experiment, high efficiency was achieved with autumn spraying of the aboveground biomass. Bímová et al. (2001) suggested cutting rhizomes into small segments followed by spraying foliage after the rhizomes regenerated, which corresponds with the reports of Jones et al. (2018). Unfortunately, excavation and cutting of rhizomes is technically arduous and not applicable at all localities. Furthermore, autumn treatment methods require higher amounts of herbicide due to the enormous amount of knotweed biomass. As a result, the evaluation of control treatment efficiency should concentrate on rhizome regeneration over the following seasons rather than shoot regrowth.

The regeneration rate of rhizomes after autumn spraying could be due to several reasons. Most probably, rhizome buds do not regenerate in late autumn, even if they are not influenced by glyphosate, due to dormancy, and they start to regenerate in the next vegetation season. Like other perennials (e.g. Liew et al., 2013), rhizomes of knotweed could be dormant as an adaptation to seasonal cold temperatures. This process can be influenced by carbohydrate storage and other physiological processes. However, further study is needed in order to understand the process of carbohydrate storage in knotweed so as to understand its processes (Klimešová et al., 2017). Carbohydrate storage is influenced by season, flowering, aboveground biomass distribution regime, and nutrient richness of the substrate. Moreover, it is a species-specific process (Martínez-Vilalta et al., 2016). The flow of assimilates and the amount of carbohydrates in storage organs directly affect glyphosate influence and the regeneration ability of rhizomes after glyphosate application. We suppose that the lower regeneration rate in autumn can be affected by a combination of plant growth characteristics during the season – amount of carbohydrates and rhizome bud regeneration dynamics. In the early season, a plant could have a lack of

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carbohydrates in its rhizomes because they have been used for spring aboveground biomass growth. The early season growth of knotweeds is extremely fast, and they form a huge amount of biomass within a short time (Lavoie, 2017). When the aboveground biomass is destroyed by glyphosate, the remaining carbohydrates are exhausted from rhizomes to form new shoots. When these new shoots are destroyed once again by glyphosate, the plant does not have enough carbohydrates to form the third cohort of new shoots and it dies. The plants could also regenerate less from rhizome buds during the late season because, in autumn, the plant is not desperate to produce aboveground biomass. Daily temperatures and light levels are declining at this time, and these are thought to be critical factors for knotweed rhizome regeneration (Bashtanova et al., 2009; Dommanget et al., 2013). Preliminary results of our further field experiments (unpublished) show that formation new shoots are higher in the following seasons after late season spraying compared to early season spraying, which has been found also in other species (e.g. Bergkvist et al., 2017).

4.4 | Knotweed control treatment and evaluation

Results from both the garden pot trial and field experiments agree with the conclusion of Jones et al. (2018) that no available control method weakens the knotweed rhizome system entirely after the one-year control treatment application. Our results indicated the highest resilience to all control methods in the hybrid *F. ×bohemica*, which is consistent with its previously observed high regeneration ability (Bímová et al., 2001; Pyšek et al., 2003).

However, the results provide evidence that glyphosate spraying significantly affects the regeneration ability of rhizomes differently than the regeneration of shoots. Shoot regeneration did not differ between seasons; rhizome regeneration rate is higher in the late season than in the early season. As a result, the evaluation of control treatment efficiency of knotweed taxa should concentrate on rhizome regeneration rather than shoot regrowth over the following seasons. We believe that the change in the common methods of evaluating efficiency, which includes measuring the

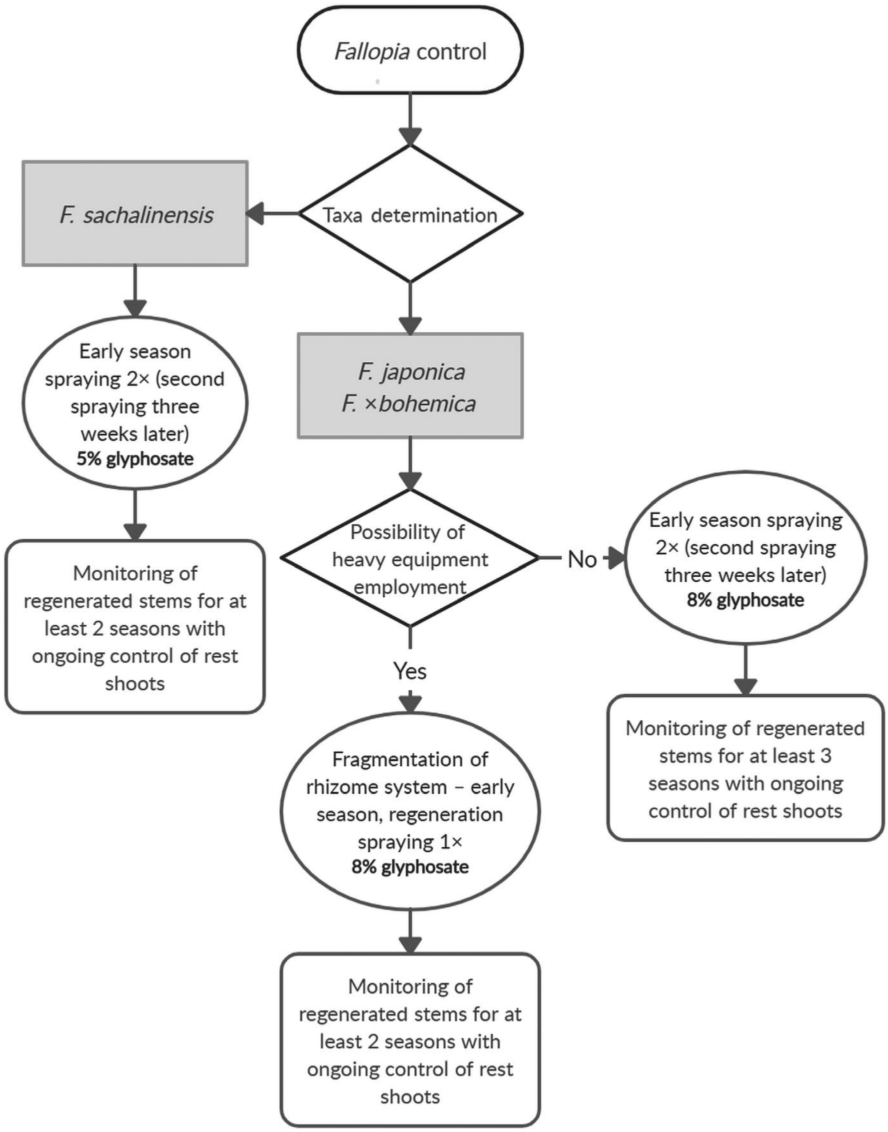

FIGURE 5 Algorithm of a recommended management procedure for successfully eliminating all taxa stands of knotweed (*Fallopia* spp.); 5%, 8% glyphosate concentration = water solution of Roundup®Active

regeneration ability of the rhizome instead of counting the aboveground shoots, will be a better parameter of evaluation.

The study results also support a taxa-specific approach to control. Below, we suggest the control algorithm for knotweed elimination based on the results of the study and previously published facts (Figure 5) (Barták et al., 2010; Bímová et al., 2001, 2003; Jones et al., 2018; Kabat et al., 2006).

We suggest stand-alone foliage spraying in mid-summer, followed by monitoring and targeting local foliage spraying in autumn as the optimal control method; it saves costs and is more environmentally friendly. The dose of glyphosate should be taxa-specific, with 5% glyphosate (3.65 kg a.i. ha^{-1}) for *F. sachalinensis* and 8% glyphosate (5.85 kg a.i. ha^{-1}) for *F.* ×*bohemica* and *F. japonica* var. *japonica*.

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CONFLICT OF INTEREST

There are no potential conflicts of interest. All persons entitled to authorship have been so named, and all authors have seen and agreed to the submitted version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

REFERENCES

Abgrall, C., Forey, E., Mignot, L. & Chauvat, M. (2018) Invasion by *Fallopia japonica* alters soil food webs through secondary metabolites. *Soil Biology and Biochemistry*, 127, 100–109. https://doi.org/10.1016/J.SOILBIO.2018.09.016

Adachi, N. (1996) Central die-back of monoclonal stands of *Reynoutria japonica* in an early stage of primary succession on Mount Fuji. *Annals of Botany*, 77(5), 477–486. https://doi.org/10.1006/anbo.1996.0058

Akaike, H. (1998) *A Bayesian analysis of the minimum AIC procedure*. New York, NY: Springer, pp. 275–280.

Barták, R., Konupková-Kalousová, Š. & Krupová, B. (2010) *Methods of elimination of invasive knotweed species (*Reynoutria *spp.)*. Moravian-Silesian Region in cooperation with ČSOP Salamandr and with financial support from the European Union. LIFE III Nature project.

Bashtanova, U.B., Beckett, K.P. & Flowers, T.J. (2009) Review: Physiological approaches to the improvement of chemical control of Japanese Knotweed (*Fallopia japonica*). *Weed Science*, 57(6), 584–592. https://doi.org/10.1614/ws-09-069.1

Beerling, D.J., Bailey, J.P. & Conolly, A.P. (1994) *Fallopia japonica* (Houtt.) Ronse Decraene. *The Journal of Ecology*, 82(4), 959. https://doi.org/10.2307/2261459

Benbrook, C.M. (2016) Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe*, 28(1), 3. https://doi.org/10.1186/s12302-016-0070-0

Bergkvist, G., Ringselle, B., Magnuski, E., Mangerud, K. & Brandsæter, L.O. (2017) Control of *Elymus repens* by rhizome fragmentation and repeated mowing in a newly established white clover sward. *Weed Research*, 57(3), 172–181. https://doi.org/10.1111/WRE.12246

Bímová, K., Mandák, B. & Pyšek, P. (2001) Experimental control of *Reynoutria* congeners: a comparative study of a hybrid and its parents. In: Brundu, G., Brock, J., Camarda, I., Child, L. & Wade, M. (Eds.) *Plant invasion: species ecology and ecosystem management*. Leiden: Backuys Publishing, pp. 283–290.

Bímová, K., Mandák, B. & Pyšek, P. (2003) Experimental study of vegetative regeneration in four invasive *Reynoutria* taxa (Polygonaceae). *Plant Ecology*, 166(1), 1–11. https://doi.org/10.1023/A:1023299101998

Blossey, B. (1999) Before, during and after: the need for long-term monitoring in invasive plant species management. *Biological Invasions*, 1(2/3), 301–311. https://doi.org/10.1023/A:1010084724526

Brock, J.H. (1995) Technical note: standing crop of *Reynoutria japonica* in the autumn of 1991 in the United Kingdom. *Preslia, Praha*, 66(1994), 337–343.

Brock, J. & Wade, M. (1992) *Regeneration of Japanese knotweed (*Fallopia japonica*) from rhizomes and stems: observation from greenhouse trials*. IXe Colloque International Sur La Biologie Des Mauvaises Herbes, 16–18 September 1992, Dijon, France, pp. 85–94.

Chmura, D., Nejfeld, P., Borowska, M., Woźniak, G., Nowak, T. & Tokarska-Guzik, B. (2013) The importance of land use type in *Fallopia* (*Reynoutria*) Japonica invasion in the suburban environment. *Polish Journal of Ecology*, 61(2), 379–384.

Clements, D.R., Larsen, T. & Grenz, J. (2016) Knotweed management strategies in North America with the advent of widespread hybrid Bohemian knotweed, regional differences, and the potential for biocontrol via the psyllid *Aphalara itadori* Shinji. *Invasive Plant Science and Management*, 9(1), 60–70.

Dommanget, F., Evette, A., Breton, V., Daumergue, N., Forestier, O., Poupart, P. et al (2019) Fast-growing willows significantly reduce invasive knotweed spread. *Journal of Environmental Management*, 231, 1–9. https://doi.org/10.1016/j.jenvman.2018.10.004

Dommanget, F., Spiegelberger, T., Cavaillé, P. & Evette, A. (2013) Light availability prevails over soil fertility and structure in the performance of Asian knotweeds on riverbanks: New management perspectives. *Environmental Management*, 52(6), 1453–1462. https://doi.org/10.1007/s00267-013-0160-3

Jones, D., Bruce, G., Fowler, M.S., Law-Cooper, R., Graham, I., Abel, A. et al (2018) Optimising physiochemical control of invasive Japanese knotweed. *Biological Invasions*, 20(8), 2091–2105. https://doi.org/10.1007/s10530-018-1684-5

Kabat, T.J., Stewart, G.B. & Pullin, A.S. (2006) *Are Japanese Knotweed (*Fallopia japonica*) control and eradication interventions effective? Systematic Review No. 21*. Collaboration for Environmental Evidence, Centre for Evidence-Based Conservation, Birmingham, UK.

Klimešová, J., Janeček, Š., Bartušková, A., Bartoš, M., Altman, J., Doležal, J. et al (2017) Is the scaling relationship between carbohydrate storage and leaf biomass in meadow plants affected by the disturbance regime? *Annals of Botany*, 120(6), 979–985. https://doi.org/10.1093/aob/mcx111

Lavoie, C. (2017) The impact of invasive knotweed species (*Reynoutria* spp.) on the environment: review and research perspectives. *Biological Invasions*, 19(8), 2319–2337. https://doi.org/10.1007/s10530-017-1444-y

Liew, J., Andersson, L., Boström, U., Forkman, J., Hakman, I. & Magnuski, E. (2013) Regeneration capacity from buds on roots and rhizomes in five herbaceous perennials as affected by time of fragmentation. *Plant Ecology*, 214(10), 1199–1209.

Majd, R., Chamanabad, H.R.M., Zand, E., Mohebodini, M., Khiavi, H.K., Alebrahim, M.T. et al (2019) Evaluation of herbicide treatments for control of wild gladiolus (*Gladiolus segetum*) in wheat. *Applied Ecology and Environmental Research*, 17(3), 5561–5570.

Martínez-Vilalta, J., Sala, A., Asensio, D., Galiano, L., Hoch, G., Palacio, S. et al (2016) Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis. *Ecological Monographs*, 86(4), 495–516. https://doi.org/10.1002/ecm.1231

Murrell, C., Gerber, E., Krebs, C., Parepa, M., Schaffner, U. & Bossdorf, O. (2011) Invasive knotweed affects native plants through allelopathy. *American Journal of Botany*, 98(1), 38–43. https://doi.org/10.3732/ajb.1000135

Nentwig, W., Bacher, S., Kumschick, S., Pyšek, P., Vilà, M. (2018) More than "100 worst" alien species in Europe. *Biological Invasions*, 20, 1611–1621. https://doi.org/10.1007/s10530-017-1651-6

Neve, P., Barney, J.N., Buckley, Y., Cousens, R.D., Graham, S., Jordan, N.R. et al (2018) Reviewing research priorities in weed ecology, evolution and management: a horizon scan. *Weed Research*, 58(4), 250–258. https://doi.org/10.1111/wre.12304

Onofri, A., Carbonell, E.A., Piepho, H.P., Mortimer, A.M. & Cousens, R.D. (2010) Current statistical issues in Weed Research. *Weed Research*, 50(1), 5–24. https://doi.org/10.1111/j.1365-3180.2009.00758.x

Parepa, M., Fischer, M., Krebs, C. & Bossdorf, O. (2014) Hybridization increases invasive knotweed success. *Evolutionary Applications*, 7(3), 413–420. https://doi.org/10.1111/eva.12139

Pysek, P., Brock, J.H., Bimova, K., Mandak, B., Jarosik, V., Koukolikova, I. et al (2003) Vegetative regeneration in invasive *Reynoutria* (Polygonaceae) taxa: the determinant of invasibility at the genotype level. *American Journal of Botany*, 90(10), 1487–1495. https://doi.org/10.3732/ajb.90.10.1487

Quinn, G.P. & Keough, M.J. (2002) *Experimental design and data analysis for biologists*. Cambridge: Cambridge University Press.

R Development Core Team (2019) *R: a language and environment for statistical computing*. (3.5.1). R Foundation for Statistical Computing. http://www.r-project.org/

Rinella, M.J., Maxwell, B.D., Fay, P.K., Weaver, T. & Sheley, R.L. (2009) Control effort exacerbates invasive-species problem. *Ecological Applications*, 19(1), 155–162. https://doi.org/10.1890/07-1482.1

Ringselle, B., Oliver, B.W., Berge, T.W., Sundheim Fløistad, I., Berge, L., Brandsæter, L.O. et al (2021) Dry weight minimum in the underground storage and proliferation organs of six creeping perennial weeds. *Weed Research*, 61(3), 231–241. https://doi.org/10.1111/WRE.12476

Rudenko, M. & Hulting, A. (2010) Integration of chemical control with restoration techniques for management of *Fallopia japonica* populations. *Management of Biological Invasions*, 1(1), 37–49. https://doi.org/10.3391/mbi.2010.1.1.06

Scott, R. & Marrs, R.H. (1984) Impact of Japanese knotweed and methods of control. *Aspects of Applied Biology*, 5, 291–296.

Skinner, R.H., van der Grinten, M. & Gover, A.E. (2012) Planting native species to control site reinfestation by Japanese Knotweed (*Fallopia japonica*). *Ecological Restoration*, 30(3), 192–199. https://doi.org/10.3368/er.30.3.192

Sołtysiak, J. & Brej, T. (2014) Invasion of *Fallopia* genus plants in urban environment. *Polish Journal of Environmental Studies*, 23(2), 449–458.

TIBCO (2017) *TIBCO Statistica®. In Computer program (13.3.0)*. San Jose, California, USA: TIBCO Software Inc.

Van Bruggen, A., He, M.M., Shin, K., Mai, V., Jeong, K.C., Finckh, M.R. et al (2018) Environmental and health effects of the herbicide glyphosate. *Science of the Total Environment*, 616–617, 255–268. https://doi.org/10.1016/J.SCITOTENV.2017.10.309

Vojík, M., Sádlo, J., Petřík, P., Pyšek, P., Man, M. & Pergl, J. (2020) Two faces of parks: sources of invasion and habitats for threatened native plants. *Preslia*, 92(4), 353–373 https://doi.org/10.23855/preslia.2020.353

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