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Long-Term Control of Hedge Bindweed (*Calystegia sepium* L.) with Single, Tank Mixture, and Sequential Applications of Glyphosate, 2,4-D, and Dicamba

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Abstract: Hedge bindweed (*Calystegia sepium* L.) is a widespread troublesome perennial weed species that has strong rhizome regenerative capacity. Four pot trials with randomised, complete block designs were conducted in 2015 to evaluate long-term control of hedge bindweed using individual, tank mixture, and sequential applications of selected herbicides. Two different formulations of *N*-(phosphonomethyl) glycine (glyphosate; isopropylamine, trimesium salts) were applied at 2000 g active ingredient (a.i.) ha^{-1}. Additionally, two synthetic auxins were applied as 3,6-dichloro-2-methoxybenzoic acid (dicamba) at 500 g a.i. ha^{-1} and the dimethylamine salt of (2,4 dichlorophenoxy)acetic acid (2,4-D) at 1000 g a.i. ha^{-1}. Tank mixtures and sequential applications (12/24 h separation) of these different herbicides were also included. Long-term control of hedge bindweed, *Calystegia sepium* L., growth was evaluated 8 months after treatments, as comparisons of shoot and rhizome growth (biomass) between untreated and treated plants. There were no differences between the two formulations of glyphosate alone, with shoot and rhizome biomass reductions of 83% and 42%, respectively. Dicamba alone inhibited shoot and rhizome biomass by 86% and 67%, respectively. By itself, 2,4-D provided the greatest reductions in shoot and rhizome biomasses, 93% and 79%, respectively. Antagonism was seen in the tank mixtures of glyphosate and dicamba or 2,4-D. Tank mixtures were generally comparable to treatments of glyphosate alone, and were less effective compared to dicamba or 2,4-D alone. The greatest reduction of bindweed rhizome biomass was for sequential glyphosate trimesium salt followed by 2,4-D 12 h later, thus showing significantly greater efficacy over glyphosate isopropylamine salt (94% vs. 84%; $p \leq 0.05$). These data for reductions of the growth of the rhizome biomass show that the sequential application of glyphosate followed by 2,4-D significantly improves long-term control of hedge bindweed.

Keywords: hedge bindweed; perennial weeds; rhizome control; weed management; herbicide efficacy

1. Introduction

Hedge bindweed (*Calystegia sepium* L.) and field bindweed (*Convolvulus arvensis* L.) (Convolvulaceae; morning glory) are two of the most troublesome perennial weeds in temperate regions [1,2]. Indeed, field bindweed is considered as one of the most serious weed species throughout the world, due to its adaptation to a wide range of habitats and cropping systems, which has already caused serious yield losses and production cost increases worldwide [3,4]. Hedge bindweed is not as widespread as field bindweed, although recent reports have shown that it has increased considerably in terms of its abundance in the USA, Europe and other parts of the world [5,6]. This expansion appears to be associated with both the increasing areas that are under reduced tillage systems, and the related changes in the use of herbicides with different spectra of activities, which tend to promote late-germinating perennial weeds [7,8].

Both of these bindweed species can spread by means of vegetative (i.e., shoots, buds, rhizomes) or generative (i.e., seeds) reproduction [1]. Their rapid regenerative capacities derive from their extensive rhizome and root systems that can store large amounts of carbohydrates, which makes their long-term control particularly difficult [9,10]. Field and hedge bindweed are closely related species [11,12]. The most important distinctive characteristic between these is that field bindweed generally spreads by roots, while hedge bindweed propagates by extensive rhizome development and above-ground runners [13]. Despite the differences in their propagation organs, they show closely analogous survival strategies in terms of rhizome and shoot sprouting, and below-ground resource storage [10]. With a lack of relevant studies on the control of hedge bindweed, the background to field bindweed will also be considered here.

Herbicide application is the most common management option for the control of hedge bindweed, although mechanical and biological methods can also be used [14]. Mechanical control with repeated soil cultivation is aimed at the depletion of the root and rhizome reserve, with success here mainly relying on the correct timing of the tillage operations and the feasibility in the cropping system [15,16]. Environmental concerns for herbicide use have also raised the need for alternative control methods. Several potential bio-control agents have shown promising results, although none have been commercialised [17,18].

A meta-analysis of the control of field bindweed within annual cropping systems reported that herbicide use dominates in the literature, and that this represents an effective management strategy up to 2 years post treatment [14]. The most common herbicides used for the control of field bindweed are *N*-(phosphonomethyl)glycine (glyphosate), 3,6-dichloro-2-methoxybenzoic acid (dicamba), (2,4 dichlorophenoxy)acetic acid (2,4-D), 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid (picloram), 3,7-dichloro-8-quinolinecarboxylic acid (quinclorac), 6-amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid (aminocyclopyrachlor), and 2-(4-isopropyl-4-methyl-5-oxo-4,5-dihydro-1H-imidazol-2-yl)nicotinic acid (imazapyr) [14,19,20]. Herbicides are, in most cases, effective for the short-term suppression of field bindweed shoot biomass; however, long-term control and eradication is generally not achieved [21].

Since its introduction in the 1970s, glyphosate has become one of the most widely used herbicides in the world. Glyphosate use has increased since it was introduced for glyphosate-resistant crops in 1996, which lead to the expansion of no-tillage and conservation cropping systems [22,23]. Glyphosate is a broad-spectrum systemic herbicide that is also used extensively for weed control in perennial fruit tree crops, and in industrial areas and other amenity and domestic situations [22,24]. An apparent lack of weed resistance to the glyphosate mode of action was believed to be a key feature of glyphosate. However, 48 weed species across six continents have now been reported to be resistant to glyphosate [25]. Although there have not been any cases of field or hedge bindweed resistance to glyphosate reported to date, the high natural tolerance to glyphosate of field bindweed, combined with the high application rate and/or repeated use of glyphosate needed to overcome this tolerance, might well increase the selection pressure and accelerate the development of resistance [26]. However, as can be observed for glyphosate, there is generally more selection for resistance in highly susceptible weeds rather than tolerant weeds [24,25].

One of the most effective groups of selective chemicals for the control of perennial broadleaf weeds and brush are the synthetic auxins. For this herbicide group, dicamba and 2,4-D have been widely used in agriculture for the past 50–70 years for the selective control of broadleaf weeds in cereal and corn fields and pastures [27,28]. Among the several cases of confirmed resistance to dicamba and 2,4-D, none of the species from the morning glory family are known to have developed resistance to these auxin herbicides [25]. However, there have been some reports of field bindweed biotypes with varied sensitivities to 2,4 D, and contrasting data on the control of field bindweed with synthetic auxins have been reported [29,30].

Several morning glory species appear to have a natural tolerance to glyphosate [26,31] and diversity in the susceptibility of field bindweed biotypes to glyphosate has been reported [32]. Tolerance to specific herbicides can usually be overcome using herbicide mixtures or sequential applications of different herbicides [33,34]. Enhanced activity on the tolerant weed species is achieved when a similarly or more effective herbicide with a different mode of action is included in the mixture or sequential treatment. There is also increasing evidence that the use of effective herbicide mixtures is a better tactic for delaying resistance than rotating different herbicidal modes of action [34]. Besides, the mixing of herbicides is generally the recommended practice, to increase the weed control spectrum, although this requires compatibility of the tank mixture components [35]. However, interactions between two or more herbicides might be antagonistic rather than additive, which will reduce or increase, respectively, the combined efficacy; their synergism might also increase the expected additive efficacy [36]. Sequential application also represents an effective tool for targeting not only troublesome weed species, but also herbicide-resistant populations [33]. This strategy is commonly used to control later-emerging weeds in systems with prolonged weed germination; however, additional herbicide follow-up might also result in better long-term suppression of hedge bindweed.

As there are few relevant data available in the literature on the management of hedge bindweed, the aim of the present study was to determine the long-term effects of herbicide control for the inhibition of hedge bindweed growth. A better understanding of hedge bindweed responses to various herbicides and the timing of their application(s) might provide knowledge for improved management strategies and more effective control of this troublesome perennial weed species, especially when considering reduced tillage systems. The objective of our study was, therefore, to determine the efficacy of glyphosate, dicamba, and 2,4-D when used as single applications and as tank mixtures and sequential applications, for the long-term inhibition of shoot and rhizome biomass of hedge bindweed (*Calystegia sepium* L.).

2. Materials and Methods

A series of greenhouse pot trials was carried out at the Faculty of Agriculture and Life Sciences in Maribor (Slovenia; 46°30′17.4″ N, 15°37′34.6″ E), from May 2015 to June 2016. A total of four trials were set up, with untreated controls and seven different treatments in each. These were arranged as randomised complete blocks with five replications. Each treatment consisted of 25 pots, and each group of five pots was considered statistically as one repetition. The herbicide treatments were applied as individual, tank mixture, and sequential applications, and included: two formulations of glyphosate of ‘Boom efekt’ (48% isopropylamine salt; IPA-glyphosate; Albaugh TKI d.o.o.,) and Touchdown system 4 (36% trimesium salt; TMS-glyphosate; Syngenta Crop Protection AG,); Banvel 480 S (48% dicamba; Syngenta Crop Protection AG); and Herbocid (460 g/L 2,4-D; Nufarm GmbH & Co KG). Moderate (glyphosate) to high (2,4-D and dicamba) herbicide doses were used in the study to obtain long-term control of hedge bindweed and to test herbicide interactions at higher herbicide rates. The details of these treatments are given in Table 1.

The plant material was collected in May 2015 from infested arable fields in the south-eastern agricultural region of Slovenia, near the city of Kidričevo (46°23′09.4″ N 15°47′00.7″ E). To minimise the variability between the biotypes, the sampling was carried out from five adjacent fields. A 50 cm × 50 cm and 25 cm deep square block was dug around the selected plants. The plants were lifted and carefully washed to remove soil from the roots and rhizome system without damaging them. After washing, the plants were placed in cold storage bins with wet sand and transported to the experimental site. The plant material from the different fields was randomised and then planted into plastic 10-L pots the same day. The pots were filled with a mixture of soil from a neighbouring field and planting substrate (1:1; v/v). High uniformity among plants was achieved by the selection of only plants with a well-developed (20 cm long) rhizome and a shoot with three to five leaves. The pots were placed in a greenhouse, watered, and allowed to grow for 5 months. Before the application of the herbicides, the development stage of the plant and the above-ground and rhizome fresh biomass were determined for the sample of 25 pots. The plant shoots were cut and the rhizomes were separated from the soil with washing. The fresh biomass of the shoots and rhizomes were determined, and the thickness and length of the rhizomes were also measured. The remaining untreated pots were left to grow in the greenhouse for the following 8 months.

Table 1. Treatments of the hedge bindweed applied in this study. For the active ingredients, glyphosates were applied at 2000 g ha^{-1}, dicamba at 500 g ha^{-1}, and 2,4-D at 1000 g ha^{-1}.

Trial	Design	Order of Application	
		First	Second
1	Control	na	na
	Single-I	IPA-glyphosate	na
	Single-II	Dicamba	na
	Tank mix	IPA-glyphosate + Dicamba	na
	Sequential-12-I	IPA-glyphosate	Dicamba
	Sequential-12-II	Dicamba	IPA-glyphosate
	Sequential-24-I	IPA-glyphosate	Dicamba
	Sequential-24-II	Dicamba	IPA-glyphosate
2	Control	na	na
	Single-I	IPA-glyphosate	na
	Single-II	2,4-D	na
	Tank mix	IPA-glyphosate + 2,4-D	na
	Sequential-12-I	IPA-glyphosate	2,4-D
	Sequential-12-II	2,4-D	IPA-glyphosate
	Sequential-24-I	IPA-glyphosate	2,4-D
	Sequential-24-II	2,4-D	IPA-glyphosate
3	Control	na	na
	Single-I	TMS-glyphosate	na
	Single-II	Dicamba	na
	Tank mix	TMS-glyphosate + Dicamba	na
	Sequential-12-I	TMS-glyphosate	Dicamba
	Sequential-12-II	Dicamba	TMS-glyphosate
	Sequential-24-I	TMS-glyphosate	Dicamba
	Sequential-24-II	Dicamba	TMS-glyphosate
4	Control	na	na
	Single-I	TMS-glyphosate	na
	Single-II	2,4-D	na
	Tank mix	TMS-glyphosate + 2,4-D	na
	Sequential-12-I	TMS-glyphosate	2,4-D
	Sequential-12-II	2,4-D	TMS-glyphosate
	Sequential-24-I	TMS-glyphosate	2,4-D
	Sequential-24-II	2,4-D	TMS-glyphosate

IPA-glyphosate, isopropylamine salt; TMS-glyphosate, trimesium salt; Sequential-12-I/II, -24-I/II, 12, 24 h between sequential applications, as glyphosate first/second; na, not applicable.

According to interviews with farmers in this area, the crops generally included in the crop rotation over the past 20 years had been maize, wheat, sugar beet, barley, and potato. The weed management practices over this period had included the application of herbicides with different modes of action, along with various cultivation operations according to conventional tillage systems. All of the farmers used herbicides according to the principles of integrated crop management. Glyphosate had been used occasionally on fallow land (i.e., cereal stubble), and synthetic auxins (e.g., dicamba, 2,4-D) had only been rarely used on cereals and maize. There had been no apparent resistance of hedge bindweed to herbicides, as reported by the farmers in the interviews.

The herbicides for the present study were applied at the beginning of October 2015, when the plants had developed 20 to 35 leaves and 35-cm- to 65-cm-long rhizomes with a thickness of 0.5 mm to 2.3 mm. The fresh biomass shoot:rhizome ratio was ~3.5/1.0, and the theoretical leaf area index was ~1.42. The plants were not at a vigorous growth stage and had partially developed flowers. The herbicides were applied with an experimental sprayer (France Technoma Euro-Pulve). The spraying capacity was 250 L ha^{-1} at an operating pressure of 3 bar. Droplets with a 125 μm to 145 μm volume median diameter were generated using the required nozzle (Teejet XR 110015). Tap water with a hardness of ~14° dH was used. The air temperature and relative humidity at the time of the herbicide application(s) were 22 °C and 68%, respectively. After the herbicide application(s), the plants were left outside the greenhouse to dry for 4 days. The pots were then placed back inside the greenhouse, and watered according to need. During the winter period, the temperature in the greenhouse was from 1 °C to 18 °C, and the plants were not exposed to freezing temperatures.

The herbicide efficacies were calculated using the method of fresh plant biomass weights [37]. The shoots and rhizomes were separated from the soil and weighed in May 2016, 8 months after the treatments. Only the green biomass of the living rhizomes was included in the samples, after the dried and necrotic parts had been removed. The reduction efficacies of the herbicide treatments (HE) against the above-ground (shoot) biomass and rhizome biomass were calculated as the ratio between the fresh weights of the plant biomasses from the untreated and treated pots, as given in Equation (1):

$$\text{HE [\%]} = ((\text{Weight of untreated control [g]} - \text{Weight of treated [g]})) / (\text{Weight of untreated control [g]}) \times 100 \quad (1)$$

the interactions between the herbicides were calculated using the method of Colby [38], as given in Equation (2).

$$E = 100 - ((100 - \text{Percent reduction by herbicide A}) \times (100 - \text{Percent reduction by herbicide B})) / 100 \quad (2)$$

the calculated values (E) for the herbicide mixtures were compared with the actual values (observed response) for the herbicide mixtures. If the actual value for the mixture was greater than the calculated value, then this indicated synergism, while a lower mixture value than the calculated value indicated antagonism. Equal values here indicated simple additive effects [37]. The same principal was also used for the analysis of interactions of herbicides for the sequential application where the calculated values (E) were compared with the actual values (observed response) for the sequential application.

A random group analysis was performed using Statgraphics Centurion XVI (2011, Statpoint Technologies, Warrenton, VA, USA), with a separate analysis performed for each experimental group. Levene's tests were used for the homogeneity of the variance. The data also underwent analysis of variance (ANOVA) using the general linear model ($\alpha = 0.05$). The variables included in the model were the blocks ($n = 5$) as random effects, and the efficacies of the herbicide treatments ($n = 7$) as fixed effects. If ANOVA indicated statistical differences, Tukey's post hoc tests were used for multiple comparisons of the herbicide treatments. The standard errors of the mean (SEM) are given to show the estimation of the variance. The differences between the IPA and TMS formulations of glyphosate were tested using Student's *t*-tests for independent sample comparisons ($\alpha = 0.05$).

3. Results

3.1. Reduction of Shoot Biomass Growth

Across the trials here, significant differences between the herbicide applications were seen for the reduction of shoot biomass ($p \leq 0.001$; Table 2), with the detailed data for shoot biomass given in Table 3.

Table 2. Statistics for sources of variation (ANOVA) for inhibition of hedge bindweed shoot and rhizome biomass growth at 8 months after applications of IPA/TMA-glyphosate and dicamba or 2,4-D (see Table 1).

Trial	Application/Residual	Statistics				
		df	Shoot Biomass		Rhizome Biomass	
			Sum of Squares	*p* Value	Sum of Squares	*p* Value
1	IPA-glyphosate and dicamba	6	872.6	<0.001	6357.8	<0.001
	Residual	24	568.9		642.3	
2	IPA-glyphosate and 2,4-D	6	910.0	<0.001	8674.3	<0.001
	Residual	24	309.4		1098.3	
3	TMS-glyphosate and dicamba	6	779.9	<0.001	6341.5	<0.001
	Residual	24	442.1		823.9	
4	TMS-glyphosate and 2,4-D	6	838.6	<0.001	7254.7	<0.001
	Residual	24	441.1		710.1	

IPA-glyphosate, isopropylamine salt; TMS-glyphosate, trimesium salt.

Table 3. Efficacy against hedge bindweed shoot biomass growth at 8 months after application(s) of IPA/TMA-glyphosate and dicamba or 2,4-D (see Table 1). No significant differences between IPA-glyphosate and TMS-glyphosate within dicamba or 2,4-D sequential combinations ($p > 0.05$; Student's *t*-tests for independent sample comparisons).

Design	Efficacy Versus Shoot Biomass Growth							
	Glyphosate/Dicamba				Glyphosate/2,4-D			
	IPA		TMS		IPA		TMS	
	(%)	Int.	(%)	Int.	(%)	Int.	(%)	Int.
Single-I	80.8	na	83.6	na	82.2	na	84.8	na
Single-II	85.6	na	87.0	na	95.0	na	91.6	na
Tank mix	90.8	–	90.6	–	92.0	–	91.8	–
Sequential-12-I	98.0	++	98.4	++	99.2	+	99.4	++
Sequential-12-II	87.8	–	85.2	–	89.8	–	85.2	–
Sequential-24-I	92.2	–	93.4	–	95.4	–	96.0	–
Sequential-24-II	89.4	–	90.8	–	89.0	–	90.8	–
SEM	2.1		1.9		1.6		1.9	

IPA, IPA-glyphosate, isopropylamine salt; TMS, TMS-glyphosate, trimesium salt; Single-I, glyphosate alone; Single-II, dicamba or 2,4-D alone; Tank mix, combinations mixed and applied together; Sequential-12-I/II, -24-I/II, 12, 24 h between sequential applications, as glyphosate first/second; na, not applicable; SEM, standard error of the means for inhibition including all treatments in the column; Int., Interactions with combined applications: −, antagonistic; +, additive; ++, synergistic (according to Colby equation; Colby, 1967); for efficacy and interactions, see Materials and Methods, Equations (1) and (2), respectively.

In the first trial (i.e., IPA-glyphosate, dicamba combinations), IPA-glyphosate followed by dicamba 12 h later showed significantly greater efficacy for the reduction of shoot biomass (98.0%) than IPA-glyphosate (80.7%) or dicamba (85.6%) alone, and dicamba followed by IPA glyphosate 12 h later (87.8%). IPA-glyphosate followed by dicamba 24 h later and tank mixtures of these two herbicides were also significantly more effective against shoot biomass (92.2%, 90.9%, respectively) compared to IPA-glyphosate alone.

In the second trial (i.e., IPA-glyphosate, 2,4-D combinations), IPA-glyphosate followed by 2,4-D 12 h later resulted in significantly greater efficacy for the reduction of shoot biomass (99.2%) compared to IPA-glyphosate alone (82.1%) and to 2,4-D followed by IPA-glyphosate 12 h or 24 h later (89.8%, 89.0%, respectively). For IPA-glyphosate alone, significantly more shoot regrowth was seen compared to the other treatments.

In the third trial (i.e., TMS-glyphosate, dicamba combinations), TMS-glyphosate followed by dicamba 12 h later showed significantly greater efficacy (98.4%) compared to TMS-glyphosate (83.6%) or dicamba (86.9%) alone, and to dicamba followed by TMS-glyphosate 12 h later (85.2%).

The results of the fourth trial (i.e., TMS-glyphosate, 2,4-D combinations) showed that TMS-glyphosate followed by 2,4-D 12 h later and 2,4-D followed by TMS-glyphosate 12 h later were significantly more effective (99.4%, 96.0%, respectively) than TMS-glyphosate (84.8%) or TMS-glyphosate followed by 2,4-D 24 h later (85.2%).

Of note here, there were no significant differences between the IPA-glyphosate and TMS-glyphosate formulations for these reductions of shoot biomass (Table 3). Here, the efficacies of the individual herbicide applications averaged 81.5% for IPA-glyphosate alone, 84.2% for TMS-glyphosate alone, 86.3% for dicamba alone, and 93.3% for 2,4-D alone. Although the differences were not significant, overall, there were greater reductions of shoot biomass associated with the TMS salt of glyphosate (Table 3). Additionally, in general, the tank mixtures were more effective compared to the individual herbicide applications, as they attained 0.2% to 11% greater reductions of shoot biomass. For shoot biomass reductions for the tank mixtures, these averaged 90.7% for glyphosate with dicamba, and 91.9% for glyphosate with 2,4-D.

Overall, the greatest reductions of shoot biomass were obtained when glyphosate was followed by dicamba or 2,4-D. Moreover, there were greater reductions compared to other treatments for both of these sequential variants with time intervals of 12 h and 24 h. However, the most efficient reduction was obtained with glyphosate followed by 2,4-D 12 h later. When this order was reversed to apply dicamba or 2,4-D followed by glyphosate, the reductions of shoot biomass showed lower efficacies, by 11.8% and 4.2% for the 12 h and 24 h sequential applications, respectively.

In the interaction analysis of the effects on shoot biomass growth of the herbicide mixtures and these sequential applications, synergistic effects were obtained for both TMS-glyphosate and IPA-glyphosate followed by dicamba 12 h later, and TMS-glyphosate followed by 2,4-D 12 h later. A simple additive effect was obtained for IPA-glyphosate followed by 2,4-D 12 h later. Antagonistic (i.e., non-additive) effects were observed for all of the other herbicide applications (Table 3).

3.2. Reduction of Rhizome Biomass Growth

For all of the trials, there were significant differences in the reductions of rhizome biomass between the herbicide applications ($p \leq 0.001$; Table 2), with the detailed data for rhizome biomass given in Table 4.

In the first trial (i.e., IPA-glyphosate, dicamba combinations), IPA-glyphosate followed by dicamba 12 h later provided significantly greater reductions of rhizome biomass (80.4%) compared to IPA-glyphosate alone (37.8%), dicamba alone (66.0%), the tank mixture of glyphosate and dicamba (50.8%), and dicamba followed by IPA-glyphosate 24 h later (66.4%). The reductions of rhizome biomass with IPA-glyphosate followed by dicamba 24 h later (70.4%) and dicamba followed by IPA-glyphosate 12 h later (73.6%) were also significantly greater compared to glyphosate alone (37.9%) and the tank mixture of glyphosate and dicamba (50.9%).

In the second trial (i.e., IPA-glyphosate, 2,4-D combinations), IPA-glyphosate followed by 2,4-D 12 h later and the reverse order of 2,4-D followed by IPA-glyphosate 12 h later both showed significantly greater reductions of rhizome biomass (84.0%, 82.8%, respectively) compared to IPA-glyphosate alone (39.4%), the tank mixture of IPA-glyphosate and 2,4-D (52.6%), and 2,4-D followed by glyphosate 24 h later (68.4%). IPA-glyphosate alone and the tank mixture of IPA-glyphosate and 2,4-D were significantly less effective compared to all of the other treatments.

In the third trial (i.e., TMS-glyphosate, dicamba combinations), TMS-glyphosate followed by dicamba 12 h later provided a significantly greater reduction of rhizome biomass (93.0%) compared to all of the other treatments. The efficacies of TMS-glyphosate followed by dicamba 24 h later (75.4%) and dicamba followed by TMS-glyphosate 12 h later (75.0%) were significantly greater compared to TMS-glyphosate alone (45.4%) or the tank mixture of glyphosate and dicamba (62.2%).

For glyphosate alone, there was significantly lower rhizome biomass suppression compared to all of the other treatments.

Finally, for the results of the fourth trial (i.e., TMS-glyphosate, 2,4-D combinations), glyphosate followed by 2,4-D 12 h later was significantly more effective (93.6%) compared to all of the other treatments. In contrast, for TMS-glyphosate alone, the reductions of rhizome biomass were significantly lower compared to all of the treatments and, for tank mixture of TMS-glyphosate with 2,4-D, the reductions of rhizome biomass were significantly lower compared to all of the sequential applications and 2,4-D alone.

Table 4. Efficacy against hedge bindweed rhizome biomass growth at 8 months after application(s) of IPA/TMA-glyphosate and dicamba or 2,4-D (see Table 1). Bold, significant differences between IPA-glyphosate and TMS-glyphosate within dicamba or 2,4-D sequential combinations ($p \leq 0.05$; Student's *t*-tests for independent sample comparisons; $\alpha = 0.05$).

Design	Efficacy Versus Rhizome Biomass Growth							
	Glyphosate/Dicamba				Glyphosate/2,4-D			
	IPA		TMS		IPA		TMS	
	(%)	Int.	(%)	Int.	(%)	Int.	(%)	Int.
Single-I	37.8	na	45.4	na	39.4	na	47.0	na
Single-II	66.0	na	67.4	na	82.0	na	75.4	na
Tank mix	**50.8**	–	**62.2**	–	52.6	–	59.0	–
Sequential-12-I	**80.4**	++	**93.0**	++	**84.0**	–	**93.6**	++
Sequential-12-II	73.6	–	75.0	–	82.8	–	79.4	–
Sequential-24-I	70.4	–	75.4	–	**71.4**	–	**82.4**	–
Sequential-24-II	66.4	–	73.6	–	68.4	–	77.6	–
SEM	2.3		2.6		3.0		2.4	

IPA-glyphosate, isopropylamine salt; TMS-glyphosate, trimesium salt; Single-I, glyphosate alone; Single-II, dicamba or 2,4-D alone; Tank mix, combinations mixed and applied together; Sequential-12-I/II, -24-I/II, 12, 24 h between sequential applications, as glyphosate first/second; na, not applicable; SEM, standard error of the means for inhibition including all treatments in the column; Int., Interactions with combined applications: –, antagonistic; ++, synergistic (according to Colby equation; Colby, 1967); for efficacy and interactions, see Materials and Methods, Equations (1) and (2), respectively.

In the IPA-glyphosate versus TMS-glyphosate comparisons using dicamba for the reduction of rhizome biomass, glyphosate followed by dicamba 12 h later and tank mixtures of glyphosate and dicamba showed significant differences ($p \leq 0.01$). Here, TMS-glyphosate was associated with greater efficacies than IPA-glyphosate. Similar benefits were seen for reductions of rhizome biomass for TMS-glyphosate over IPA-glyphosate in the 2,4-D trials, with significant differences for glyphosate followed by 2,4-D 12 h ($p \leq 0.05$) and 24 h ($p \leq 0.01$) later, and 2,4-D followed by glyphosate 24 h later ($p \leq 0.05$).

As can be seen from the efficacy data in Table 4 for the reduction of rhizome biomass, the averages for the individual herbicide applications were 38.6% for IPA-glyphosate, 46.2% for TMS-glyphosate, 66.7% for dicamba, and 78.7% for 2,4-D. On average, the tank mixtures were less effective compared to the individual herbicides alone, at 56.5% for glyphosate and dicamba, and 55.8% for glyphosate and 2,4-D. Additionally, in the overall comparisons of the reduction of rhizome biomass associated with IPA-glyphosate and TMS-glyphosate use with dicamba and 2,4-D, these showed greater benefits for TMS-glyphosate (18.3%, 10.8%, respectively).

The sequential applications of these herbicides showed the greatest reduction of rhizome biomass. Overall, the greatest efficacies were seen for glyphosate followed 12 h later by dicamba (93.0%) or 2,4-D (93.6%). Conversely, if dicamba or 2,4-D were followed by glyphosate, the final rhizome biomasses were greater (19.3%, 15.2%, respectively). On average, for the sequential applications of TMS-glyphosate versus IPA-glyphosate with dicamba and 2,4-D, TMS-glyphosate showed greater reductions of rhizome biomass, with increases of 8.2% and 7.9%, respectively, over IPA-glyphosate. Furthermore, the comparisons of the efficacies that showed significant differences for the reduction of rhizome biomass between IPA-glyphosate and TMS-glyphosate were always in favour of TMS-glyphosate, as it was 12.6% greater when followed by dicamba 12 h later, 9.6% greater when followed by 2,4-D 12 h later and, finally, 11.0% greater when followed by 2,4-D 24 h later. Furthermore, TMS-glyphosate was also more efficient for the reduction of rhizome biomass even for the 24 h sequential applications. Differences in reductions were also seen for these efficacies between 12 h and 24 h for 2,4-D followed by IPA-glyphosate (14.4%) compared to TMS-glyphosate (1.8%). For dicamba followed by IPA-glyphosate or TMS-glyphosate, a similar reduction of the efficacies for the applications 12 h after to 24 h after were seen (IPA: 7.2%; TMS, 1.4%).

The results of the interaction analysis of the effects on rhizome biomass of the herbicide mixtures and these sequential applications showed synergistic effects for IPA-glyphosate followed by dicamba 12 h later and TMS-glyphosate followed by dicamba or 2,4-D 12 h later. Antagonistic interactions were observed for all of the other herbicide applications (Table 4).

4. Discussion

Due to the lack of relevant studies on the control of hedge bindweed in particular, we include here a reference to the more studied field bindweed for comparisons and discussion. As these two bindweeds are closely related, we would expect small physiological and morphological variations affecting the biological efficacy of the tested herbicides. However, this presumption remains speculative, particularly for glyphosate, as significant differences in the natural tolerance of field bindweed compared to the glyphosate-susceptible Japanese false bindweed (*Calystegia hederacea*) were reported [26]. Differences in tolerance to glyphosate among the biotypes of field bindweed have also been reported [39], which indicated a high variability of tolerance to glyphosate among these species.

Modern production systems with conservation and no-tillage practices are favouring specialisation and weed management simplification, especially for herbicide-resistant crops. Such simplification for weed control can be seen in the abandonment of good weed management practices (rotation of herbicide active ingredient) and increasing reliance on glyphosate [40]. The chemical control of hedge bindweed is therefore also shifting towards continual selection pressure from the same class of herbicide, and such actions usually contribute to rapid shifts in weed species populations or resistance development [40–42]. Additionally, a lack of genuine new modes of action of herbicides and a decline in the available active ingredients [43] have increased the dependence on only a few herbicide groups for the control of hedge bindweed. To delay the development of resistance to the few remaining herbicides, tank mixtures or sequential applications can be recommended [33]. However, the effectiveness of these methods for the control of hedge bindweed are not known.

The data from the present study show that a relatively high reduction of shoot biomass growth 8 months after the application of these herbicides can be achieved, generally regardless of whether they are applied individually, in mixtures, or sequentially. These findings are in agreement with a number of studies reporting on effective (>90%) above-ground biomass suppression of the closely related field bindweed across different herbicides [21,32,44–46]. Limitations to the reduction of the above-ground biomass of hedge bindweed obtained by glyphosate over longer time periods were seen in the present study. Applications of glyphosate alone and sequential applications with glyphosate follow-up were less effective for the reduction of the above-ground growth of hedge bindweed 8 months after their application, compared to dicamba and 2,4-D applied alone or following glyphosate.

The simple assumption of the successful control of hedge bindweed based on the effective suppression of the above-ground biomass is usually not reliable for such perennial weeds with extensive rhizome systems. Even after complete destruction of the above-ground shoots, the stored reserves in the rhizomatous root system enables the development of regenerative shoots, even after relatively long periods of time. For the effective control of hedge bindweed with herbicides, good translocation of herbicide and high biological efficacy to the rhizome system are thus needed. However, as demonstrated for field bindweed, even when these criteria have been met, continual treatments over longer periods are usually required to effectively overcome this regeneration from the root system [14]. In the present study, herbicides and rates were selected with the aim to achieve the long-term rhizome reduction of hedge bindweed. However, there were live rhizomes in most of the pots after the removal of the soil even when there was little or no above-ground growth seen. Indeed, there were weak correlations between the control of the above-ground versus rhizome biomasses after 8 months, even for the herbicides that resulted in almost no shoot growth 8 months after their application (data not shown).

The greatest rhizome biomass reductions at 8 months after herbicide applications were for the sequential applications of TMS-glyphosate followed by 2,4-D or dicamba 12 h later. Indeed, synergistic interactions were obtained for these sequentially applied dicamba or 2,4-D at 12 h (although only after TMS-glyphosate), while there were antagonistic interactions for the other sequential applications. Similarly, greater control of field bindweed was reported for sequential herbicide applications compared to tank mixtures or the herbicides applied alone in other studies [21,47]. Furthermore, the improved control of grasses and broadleaf weeds with glyphosate used in sequential applications was also reported [48–50]. The reductions of the rhizome biomass of hedge bindweed in the present study were higher compared to those for field bindweed in the studies by Stone et al. and Hoss et al. [21,46]. This can be partly attributed to the limitations of the pot experiments, where the ratio between the rhizome and shoot biomass differs from that seen under field conditions, which was here in favour of the above-ground biomass. Consequently, the herbicide concentrations that entered the rhizome tissues might have been higher here, thus apparently enhancing the herbicide performance.

The data here for the sequential applications of these herbicides suggest that, after the initial application of glyphosate, sufficient membrane integrity was maintained in the treated leaves for at least 24 h, to thus sustain the translocation flow to allow the plants to take up and translocate the sequentially applied auxins to the rhizomes. However, the reductions of efficacy seen here show that there might have been decreased indices of uptake or translocation of dicamba or 2,4-D when applied 24 h after the glyphosate. As glyphosate can inhibit the transport of auxins and enhance auxin oxidation [24], the translocation of dicamba or 2,4-D applied 24 h later might have been more affected than when applied only 12 h after glyphosate, which would have resulted in the lower reductions of rhizome biomass observed. When dicamba or 2,4-D was applied first with the later application of glyphosate, even smaller reductions of rhizome biomass were observed. As dicamba and 2,4-D can rapidly disrupt the plant carbon flow [51,52], the translocation of sequentially applied glyphosate (which is translocated using this mechanism) might well be affected by this. This is supported in a study for the control of the annual broadleaf weed species kochia (*Kochia scoparia* (L.) Schrad.), where the plant translocation system was inhibited when glyphosate and dicamba were applied as a tank mixture [53].

For the rhizome system, the individual herbicide treatments were generally less effective compared to the sequential applications. Dicamba and 2,4-D showed greater rhizome biomass reductions compared to glyphosate. Several studies have partly attributed variations in the herbicide performances to differences in agro-climatic conditions, affecting the uptake and translocation of herbicides [20,47]. Regardless of the salt formulation, when glyphosate was applied alone, it showed the lowest reductions of hedge bindweed rhizome biomass across these trials. The application of a similar dose of 2.24 kg ha^{-1} glyphosate reduced the infestation of field bindweed by 24% under conditions of low humidity, and by 60% under humid conditions [39], which again indicates that environmental conditions have an important effect on the efficacy of glyphosate. One of the weaknesses of glyphosate is its lower efficacy against broadleaf weeds when applied at low and moderate doses [24]. With the moderate dose of glyphosate used in the present study, this might explain the relatively low reductions of biomass achieved. However, even with higher doses of glyphosate applied alone, consistent efficacy against field bindweed has not been maintained across a number of studies [15,21]. DeGennaro and Weller [32] explained the contrasting data in their study according to the natural variation of susceptibility to glyphosate across field bindweed biotypes. Indeed, they needed a four-fold higher rate of glyphosate treatment (4.5 kg ha^{-1}) to show efficacy against glyphosate-tolerant populations. The reasons for such differences in susceptibility to glyphosate were further elaborated on, with detailed discussion of the cellular mechanisms that influence differential glyphosate sensitivities in the bindweed field biotypes [26,39]. Additionally, the amount of glyphosate transported from the source leaves to the sink tissues can be self-limiting, because the glyphosate mechanism of action inhibits the transport of assimilates [54]. Other related species of hedge bindweed, such as *Ipomoea* spp., can also show higher natural tolerance to glyphosate [55].

Some differences among the glyphosate formulations were observed in the present study, with greater efficacy for glyphosate in the form of its trimesium salt. These differences were observed across the four trials, and they cannot simply be explained by random variability or by sampling error. Potentially, the uptake and translocation of these two glyphosate salts differ, allowing one to have greater efficacy against the hedge bindweed rhizomes. Based on these findings, it would appear that for the TMS formulation there would have been slower collapse of the cell photosystem activity, allowing translocation to be sustained for longer. Longer cell activity and translocation to the rhizome system with TMS-glyphosate would also explain the greater efficacy of the sequentially applied dicamba and 2,4-D. However, we are not able to confirmation this possibility, or indeed other explanations, here and there remains no definitive report in the literature.

The tank mixtures of glyphosate with dicamba and 2,4-D in the present study showed antagonistic effects that resulted in a lower reduction of rhizome biomass compared to the individual applications of dicamba and 2,4-D, and their sequential applications in general. The efficacies of the tank mixtures against rhizome biomass were only comparable to glyphosate applied alone. This effect was not seen for the reductions in above-ground biomass, with similar antagonistic interactions seen. Contrasting results of the performance of glyphosate and synthetic auxins as tank mixtures were reported [20,52,56,57]. In the studies, these herbicide mixtures showed either synergistic or antagonistic effects when applied to field bindweed and other perennial weeds. These inconsistent results might also indicate that the interactions between these two herbicide groups are weed species specific. Antagonism between glyphosate and 2,4-D was reported against wild oat (*Avena fatua* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and johnsongrass (*Sorghum halepense* L.) [56,58]. Antagonistic interactions between glyphosate and dicamba were also seen against kochia and johnsongrass [53]. Furthermore, antagonistic interactions between glyphosate and dicamba were observed on both glyphosate- and dicamba-resistant and susceptible kochia, as a result of the decreased translocation of these two herbicides [53]. Conversely, glyphosate supplemented with dicamba had positive effects, with a greater reduction of horseweed seen [59]. Green [60] noted that reduced rates of herbicides used in tank mixtures can also produce synergistic interactions, although this was not confirmed for hedge bindweed in the present study.

The mixing of herbicides is generally the recommended practice to reduce herbicide use while maintaining weed infestation at acceptable levels [60]. Furthermore, if improved efficacies of mixtures against weeds are obtained with reduced doses of the component herbicides, crop production can be more cost effective [36,61]. The use of the available herbicides as tank mixtures is considered as one of the proactive approaches to address the development of resistance in broadleaf weeds [62]. It has also been reported that the mixing of herbicides with different modes of action can overcome specific bindweed tolerances to selected herbicides [36]. Further increases in the use of herbicide mixtures against weeds is expected, with the introduction of multiple herbicide-resistant crops with dicamba or 2,4-D plus glyphosate and/or glufosinate resistance [63]. Compared to the annual rotation of herbicides with different modes of action, there are also some indications that herbicide mixtures are more effective in preventing the development of resistance [33,64]. However, based on the results of the present study, tank mixtures of glyphosate and dicamba or 2,4-D would not be recommended against hedge bindweed. The sequential application of glyphosate with dicamba or 2,4-D 12 h later provided the greatest efficacy of hedge bindweed here, and so this should be an effective method to reduce even heavy infestations of hedge bindweed. However, our findings from pot experiments should also be tested under field conditions to validate this.

5. Conclusions

This study shows that hedge bindweed can be effectively managed without glyphosate, as dicamba or 2,4-D applied alone provided greater efficacy against rhizome biomass compared to glyphosate alone. In treatments with the sequential application of glyphosate followed by dicamba or 2,4-D, further reductions in rhizome biomass were achieved. This can reduce the necessary applications and herbicide input in the long term, especially in the conservation and no-tillage systems mainly relying on chemical weed control of hedge bindweed with glyphosate. Such an approach can be viable for spot treatments or for areas under extreme infestation with hedge bindweed, where repeated treatments over longer periods of time will be needed. As well as improved efficiency, this strategy also provides preventive anti-resistance mitigation measures, as alternative modes of action are included in these sequential treatments. Antagonism was observed for the tank mixtures of glyphosate and dicamba or 2,4-D, and so these mixtures were less effective compared to individual applications of dicamba or 2,4-D. Although these applications were only made during the autumn here, early tillage and mechanical methods should also be a part of integrated management strategies for hedge bindweed, which will further improve the long-term control of this troublesome perennial weed species.

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