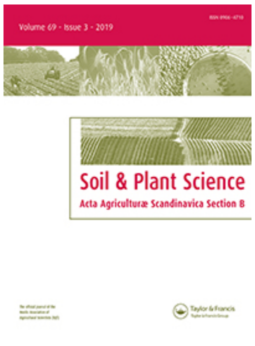

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Growth and development of *Cirsium arvense* in relation to herbicide dose, timing of herbicide application and crop presence

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ABSTRACT
The aim of this study is to assess control effects of 2-methyl-4-chlorophenoxyacetic acid (MCPA) dosage, application timing and crop presence on the weed *Cirsium arvense*. Swedish farmers are recommended to control *C. arvense* chemically when most shoots are 10-20 cm tall, and mechanically at the compensation point (CP). Recent studies have shown that the CP occurs before shoots reach the three-leaf stage. We hypothesised that (i) herbicide application near the three-leaf stage gives the strongest control, (ii) crop presence increases herbicide effects, and (iii) a 50% herbicide dose gives the same effect as 100%. Treatments of the pot experiment consisted of MCPA 750; 0%, 50% and 100% of the recommended dose applied at leaf stages 3-8, with and without barley. The strongest control was obtained at four leaves and a maximum shoot height of 13 cm, using the recommended dose and with spring barley. In a field population, a maximum shoot height of 13 cm corresponded to a medium height of 6 cm. The 50% dose gave poorer control. Spraying with the recommended dose at the four-leaf stage reduced the development of *C. arvense* most effectively. Based on this, we recommend that herbicide spraying should be performed at earlier leaf stages/median heights than previously recommended.

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Biomass production; Canada thistle; MCPA; phenology; spring barley; weed control

Introduction

Cirsium arvense is a troublesome and persistent perennial weed in the temperate regions of the world (Donald 1994; Fogelfors & Lundkvist 2008; Brandsæter et al. 2010). The species spreads by seeds and by vertical and horizontal roots, giving rise to aerial shoots (Bakker 1960; Moore 1975; Nadeau & Van den Born 1989; Verwijst et al. 2017). By its extensive and fast-growing root system, *C. arvense* competes successfully with other plant species in extracting water and nutrients (Donald 1994). In agricultural systems, *C. arvense* reduces yields in both annual and forage crops significantly (Moore 1975).

Due to its economic impact on crop production, the need to control *C. arvense* effectively is therefore strong (Tiley 2010). Since the introduction of herbicides, chemical control has been the major tool for control of *C. arvense* (Håkansson 2003). Phenoxy herbicides like 2-methyl-4-chlorophenoxyacetic acid (MCPA) are widely used in cereals and grassland, giving an immediate rapid but limited long-term effect on *C. arvense* (Thind 1975). However, environmental and societal considerations have been raised regarding the use of herbicides and other pesticides during the last decades (Tilman et al. 2002; Kudsk & Streibig 2003). In 2009, an EU directive was implemented which promotes the more efficient use of herbicides coupled with mechanical and cultural control, i.e. integrated pest management (Anonymous 2009). Since then, the interest in using reduced herbicide doses and non-chemical control methods has increased. Special attention has been paid to organic farming where cultural and mechanical control methods are used to suppress *C. arvense* and other troublesome weeds, thereby favouring the competitive ability of the crop (Melander et al. 2005; Graglia et al. 2006).

In early development stages, *C. arvense* is rather susceptible to shading and competition for light from neighbouring plants in crop stands (Tiley 2010). The *C. arvense* plants produce fewer shoots with less flowers as compared to plants growing in an unshaded environment (Bakker 1960). Well-established and fast-growing cereal crops may, therefore, suppress the growth and development of *C. arvense* substantially. By combining management practices such as competitive crops and soil cultivation with reduced herbicide rates, the odds of successful weed control increases (Boström & Fogelfors 1999; Zhang et al. 2000). In a study of long-term effects

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of different herbicide application strategies, Boström and Fogelfors (2002) demonstrated the potential of reducing herbicide doses against annual weeds without lowering yields in spring-sown cereals. Salonen (1992) showed that a reduction of MCPA/mecoprop and MCPA/fluroxypyr mixtures to half of the recommended dose gave effective control of annual weeds in spring cereals. However, Fogelfors and Lundkvist (2008) demonstrated that reduced doses of MCPA in comparison to recommended dose may significantly increase the above-ground weight of the perennial weed species *C. arvense* and *Sonchus arvensis* L.

To obtain strong control effects, direct weed control measures should be performed when *C. arvense* is most susceptible to them. Usually, perennial weeds are considered to be most sensitive to spraying between budding and flowering stages, i.e. the peak period of carbohydrate transfer downwards and metabolic activity in the roots (Wiese & Rea 1962; Hodgson 1970, 1973, Chodova & Mikulka 1986). Tworkoski (1992), however, speculated that spraying would be most efficient at the elongation stage since more photoassimilates in *C. arvense* were moved to roots at this stage compared with bud and flowering stages. In Sweden, spraying with MCPA is recommended when the majority of the *C. arvense* shoots have reached a height of 10–20 cm (Widén & Johansson 2015, 2016; Jordbruksverket 2017a, 2017b) with adequate foliage present, but before flowering (Anonymous 2017a). However, as foliage-applied herbicides need to be absorbed by leaves, and the relation between leaf area and shoot height can be affected by factors such as ecotype and crop competition; leaf area, as reflected by leaf number, might be a more direct indicator than shoot height. Leaf number also is used as a diagnostic tool to determine the timing of mechanical control of *C. arvense* (Dock Gustavsson 1997; Håkansson 2003; Verwijst et al. 2018). When *C. arvense* has reached its minimum weight of the below-ground structure (compensation point, CP) in the spring, it is considered to be most vulnerable for a disturbance. Earlier experiments have indicated that CP coincided with an average of 8–10 leaves per shoot (Dock Gustavsson 1997). However, recent studies have shown that CP seems to occur before the three-leaf stage is reached (Verwijst et al. 2018). This indicates that mechanical control should be performed at earlier phenological stages than previously recommended to prevent assimilation of carbohydrates which eventually may be used for regeneration. For the same reason, *C. arvense* may also be most sensitive for chemical weed control at early leaf stages, provided that the leaf area is sufficiently large to warrant absorption of the herbicide.

The aim of this study was to assess the effects of timing of herbicide application, herbicide dose and crop presence on growth and development of *C. arvense*. The effect of a control method is strongly dependent on targeting *C. arvense* at its most vulnerable developmental stage. This is assumed to be at the CP which occurs just before the three-leaf stage in *C. arvense* (Verwijst et al. 2018). We, therefore, hypothesise that herbicide application close to a stage of three leaves gives the strongest control effect on *C. arvense*. Since *C. arvense* is susceptible to competition for light at early developmental stages, we expect that the presence of a crop will increase the control effect from the herbicide treatments. We also hypothesise that a reduced herbicide dose gives the same control effect as the recommended dose.

Material and methods

Experiment

During June–September 2014, a pot experiment was conducted outdoors in a net enclosure at Ultuna close to Uppsala, Sweden (59°48′N, 17°39′E) to assess effects of herbicide dose, the timing of herbicide application and presence of a crop. In June, mean temperature and accumulated radiation were lower while accumulated precipitation was higher compared with the period 1960–1990, while the opposite weather conditions occurred during July–September (Table 1). The pots (experimental units) had a volume of 0.012 m^3 and a surface area of 0.064 m^2. They were filled with soil and irrigated just before planting. The soil consisted of 85% moderately composed peat, 15% sand, total N content of 0.057 kg m^{-3}, and NPK proportion of 2:1:2 (Hasselfors Garden AB, Sweden).

Plant material

The plant material used was *C. arvense* and spring barley (*Hordeum vulgare* L. var. SW Waldemar). Root material of *C. arvense* was harvested from a plant bank kept at

Table 1. Mean monthly values of air temperature, radiation and precipitation at the experimental site in June–August 2014, compared with average climate for the period 1960–1990 (Anonymous, 2017b).

	Mean temperature (°C)		Radiation (∑ MJ m^{-2})		Precipitation (∑ mm)	
	2014	Average 1960–1990	2014	Average 1960–1990	2014	Average 1960–1990
June	13.6	15.0	550.8	619.6	71.2	45.5
July	20.0	16.3	665.8	571.2	24.0	71.8
August	16.7	15.1	457.4	441.9	93.0	69.9
September	12.0	10.8	314.8	259.1	54.4	56.3

Ultuna. The plant bank was established in 2008, when *C. arvense* roots were collected from an organically managed field near Uppsala. From October until June each year, the plant bank was stored in pots with a soil volume of 0.012 m^3 in a dark cold store at a temperature of +2°C to +4°C and grown over summers in outdoor conditions in the same pots, fertilised with 70 kg N ha^{-1} $year^{-1}$ and regularly irrigated.

One day before planting (19 June), *C. arvense* roots were harvested from the plant bank. The root material was washed and roots with a diameter of approximately 3–5 mm were cut into pieces, each with a fresh weight of 2.4 g. Root pieces with at least two viable buds were selected.

Experimental design

In the experiment, a randomised block design with five blocks was used. The experimental factors were herbicide treatment (control, 50%, and 100% of recommended dose), the timing of herbicide application and crop presence (with and without spring barley). The timing of herbicide spraying followed six phenological development stages of *C. arvense* (3–8 leaves).

In advance, it was randomly determined which experimental units should be (i) planted with spring barley, and (ii) sprayed at what phenological stage. At each herbicide treatment, corresponding control treatments were harvested to assess growth and development of *C. arvense*. As controls at final harvest, another 10 units (5 units with *C. arvense* and 5 units with *C. arvense* + spring barley) were included in the experiment. In total, the trial consisted of 190 experimental units (5 blocks × 2 levels of competition × 3 levels of herbicide treatment × 6 leaf stages, and 10 control units at final harvest).

Planting and fertilisation

On 20 June, one root fragment per pot was planted in each of the pots at a depth of about 5 cm. In half of the experimental units, spring barley was sown when the first shoot of *C. arvense* per experimental unit had emerged. Planting depth was 3 cm and the seeding rate was 400 seeds m^{-2}, i.e. 25 seeds pot^{-1}. The mixed stands (*C. arvense* + spring barley) were separated from the pure stands (*C. arvense*) to avoid effects of shading. The pots were irrigated after planting and the soil was kept moist during the whole growing season.

On 30 June, the pots were fertilised with Blomstra (Cederroth International AB, Sweden) at an application rate of 60 kg N ha^{-1}. Three to four weeks after planting, *C. arvense* was attacked by aphids (*Aphidoidea* spp.) and therefore sprayed with the insecticide imidacloprid (Provado Calypso Insektsspray, Bayer Garden, Sweden).

Herbicide application

During 16 June to 14 July, the herbicide treatments were performed indoors at Ultuna in a closed ventilated spray chamber set to resemble field spray treatments (Kristensen 1992). Spraying was done with a moving boom equipped with two hydraulic nozzles (Hardi ISO F-110, 025 Standard Flat Fan nozzles). The spray boom speed was set to 6.0 km h^{-1} and spray pressure to 300 kPa which gave a spray volume of 140 L ha^{-1}. The distance between the nozzles and the top of plants was adjusted to 50 cm.

The plants were sprayed with MCPA in the commercial formulation MCPA 750 [750 g active ingredient (a.i.) L^{-1}, NuFarm, Australia]. Herbicide solutions were prepared in deionised water to 750 g a.i. ha^{-1} (100% of recommended dose) and 375 g a.i. ha^{-1} (50% of the recommended dose). Before and after each herbicide treatment, nozzle spray rate was controlled by spraying distilled water on containers placed in the spray chamber. The amount of water was measured and spray volume was calculated.

Herbicide spraying was performed with respect to the phenology of *C. arvense*. Leaf number of the largest shoot in each bucket was recorded daily. When the largest shoot had reached the predetermined phenological stage, i.e. when the last developed leaf was 5 cm or longer and unfolded, spraying was performed. Herbicide treatments were done on 16, 20, 24, 29 June, and 4 July at phenology 3–7 leaves, respectively. Herbicide treatment at the eight-leaf stage was performed on 8 July in pots with *C. arvense*, and on 14 July in pots with *C. arvense* + spring barley. The difference in treatment time at the eight-leaf stage of *C. arvense* was due to a delay in its phenological development, which became visible at that stage, and likely caused by crop presence.

Following spraying, treated plants were kept indoors at +20°C to ensure translocation of the herbicide into the plants. After 5–6 h, they were returned outside to the net enclosure.

Harvests

Harvest of controls at spraying. When herbicide treatments had been performed, the corresponding control treatments were harvested. The first harvest was done when spraying was performed at the three-leaf stage of *C. arvense*. In each of the harvested pots, the height of the largest shoot was measured and the phenological stage of each *C. arvense* shoot was recorded. The *C. arvense* plants were partitioned into above-ground parts (leaves and stems) and below-ground parts [below-ground shoots, old root (originally planted root fragment), new roots and fine roots]. The samples were dried at 105°C for 24 hours and weighed. No measurements were performed on spring barley. The same procedure was used for harvests at phenological stages of 4–8 leaves.

Total plant weight per experimental unit was calculated by adding the weights of above-ground and below-ground parts. Average phenological stage per experimental unit was calculated by adding the phenological stage for each shoot and dividing by the number of shoots.

Final harvest. On 11 September, all herbicide treated experimental units and the additional control units were harvested following the procedure described above.

Shoot height measurements

To estimate relationships between median and maximum shoot heights, *C. arvense* shoots were measured at the beginning of June 2017 in a field experiment near Uppsala, Sweden. The experiment was sown with spring barley and contained naturally occurring *C. arvense* populations. In each of the 24 experimental plots, shoot heights were measured within an area of 20 m^2. For further information on this experiment, see Tavaziva (2017).

Table 2. ANOVA results, showing the effects of crop presence, spraying, dose, phenology, dose × phenology and dose × crop × phenology on below-ground weight (g $plant^{-1}$), total weight (g $plant^{-1}$), maximum shoot height (cm), average shoot height (cm) and average phenology (no leaves $shoot^{-1}$) of *C. arvense* at harvest.

Dependent variable	Experimental factor (level)	d.f.	*P**
Below-ground weight (g $plant^{-1}$)	Crop (yes, no)	1	<.0001
	Spraying (yes, no)	1	.6755
	Dose (50%, 100%)	1	.0032
	Phenology (3–8 leaves)	5	.0702
	Dose × phenology	5	.2992
	Dose × crop × phenology	12	.0248
Total weight (g $plant^{-1}$)	Crop (yes, no)	1	<.0001
	Spraying (yes, no)	1	.0515
	Dose (50%, 100%)	1	.0020
	Phenology (3–8 leaves)	5	.0125
	Dose × phenology	5	.6011
	Dose × crop × phenology	12	.0559
Maximum shoot height (cm)	Crop (yes, no)	1	.0290
	Spraying (yes, no)	1	<.0001
	Dose (50%, 100%)	1	.0002
	Phenology (3–8 leaves)	5	<.0001
	Dose × phenology	5	.0711
	Dose × crop × phenology	12	.6124
Average phenology (no leaves $shoot^{-1}$)	Crop(yes, no)	1	.2892
	Spraying (yes, no)	1	.0994
	Dose (50%, 100%)	1	.0003
	Phenology (3–8 leaves)	5	.3150
	Dose × phenology	5	.7982
	Dose × crop × phenology	12	.3007

Note: d.f.: numerator degrees of freedom.
**P*: probability value for type 3 tests of fixed effects.

Statistical analyses

Biomass, shoot height and phenology

To make the variance homogenous, below-ground weight, total weight, maximum shoot height and average phenology of *C. arvense* were square-root transformed prior to the analysis of variance.

The effects of treatments on below-ground weight, total weight, maximum shoot height and average phenology of *C. arvense* at harvest were evaluated by using a mixed model with fixed effects of block, crop (yes/no), spraying (yes/no), herbicide dose (50% and 100% of recommended dose), phenology at spraying (3–8 leaf stages) and their interactions. Differences in the least square means were tested using the Lsmestimate function (SAS Institute 2011).

The combined effects of the crop, herbicide dose and phenology on below-ground weight, total weight, maximum shoot height and average phenology of *C. arvense* at harvest were evaluated by using a linear model containing fixed effects of treatment (crop, dose and phenology combined) and block. The analysis was performed for the four treatment combinations: (i) 100% dose with a crop, (ii) 100% dose without a crop, (iii) 50% dose with a crop and (iv) 50% dose without a crop. The Dunnet–Hsu test was used to do a pairwise comparison between the control and each of the phenological stages (3–8 leaf stages) (SAS Institute 2011).

The effects of crop and herbicide dose on changes in below-ground weight, total weight, maximum shoot height, and average phenology over time (between spraying and harvest) were assessed by linear models containing fixed effects of comparison (1: dependent variable at spraying, 2: dependent variable at harvest), phenology (3–8 leaf stages), block and the interaction comparison × phenology. The analyses were performed for the treatment combinations: (i) 100% dose with a crop, (ii) 100% dose without a crop, (iii) 50% dose with a crop and (iv) 50% dose without a crop. Tukey's test was used to do a pairwise comparison among the phenological stages.

Models were fitted using the MIXED and general linear models procedures of the SAS System (SAS Institute 2011). Linear regression analysis was used to establish relations between median and maximum shoot heights (Dell Inc. 2015).

Results

Below-ground biomass

Below-ground biomass at harvest

The below-ground weight of *C. arvense* at harvest was significantly affected by crop, dose and the interaction dose × crop × phenology (Table 2). The presence of spring barley reduced below-ground biomass from 27.6 g plant^{-1} to 4.6 g plant^{-1}, the reducing effect being larger at a later phenology (leaf stages 5–8). No significant differences in below-ground biomass were observed between control plants (14.0 g plant^{-1}) and plants sprayed with herbicide doses 50% (14.9 g plant^{-1}) and 100% (11.9 g plant^{-1}), respectively. However, the difference in below-ground biomass between the 50% dose and 100% dose was significant.

When evaluating the effects of crop (with or without spring barley) and herbicide dose (50% or 100% of recommended dose) sprayed at different developmental stages of *C. arvense*, i.e. the interaction dose × crop × phenology, significant effects on below-ground weight were obtained when *C. arvense* grew with spring barley and was sprayed with full dose (100%) at three- and four-leaf stages (Figure 1(A)). Compared with the control (5.5 g plant^{-1}), below-ground weight was reduced to 1.5 g plant^{-1} (three-leaf stage) and 1.3 g plant^{-1} (four-leaf stage), respectively. No significant effects were observed on below-ground weight in the other treatment combinations: 100% dose + no crop; 50% dose + crop, and 50% dose + no crop.

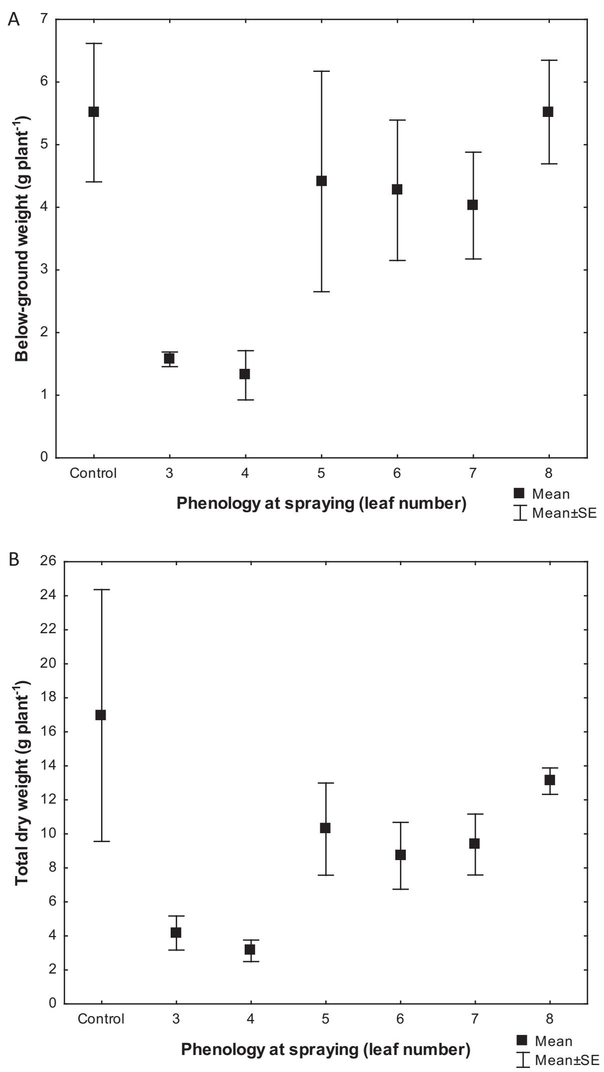

Figure 1. *Cirsium arvense* (g plant^{-1}) (mean ± SE) on raw data (A) below-ground dry weight, and (B) total dry weight at harvest (■) grouped by phenology at spraying and categorised by herbicide dose (100% with spring barley). Control was used to do a pairwise comparison with each of the phenological stages of 3–8 leaves at harvest.

Below-ground biomass at spraying and at harvest

When assessing biomass growth over time (between spraying and harvest) for each phenological stage, no significant increase in biomass was found when *C. arvense* grew with spring barley and was treated with 100% dose (Figure 1(A)). Similar results were observed when *C. arvense* was treated with 50% dose and grew with spring barley. Lowest below-ground weight at harvest (2.2 g plant^{-1}) was found when the plants were treated at the three-leaf stage. In the two other treatment combinations (100% dose + no crop, and 50% + no crop), below-ground weight increased significantly over time for all phenological stages. At harvest, below-ground weight was on average 21.7–37.5 g plant^{-1}.

Total plant biomass

Total plant biomass at harvest

Total plant weight of *C. arvense* at harvest was significantly affected by crop, dose and phenology (Table 2). The presence of spring barley significantly reduced plant biomass to 11.8 g plant^{-1} compared with plants growing without a crop (69.2 g plant^{-1}). Treatment with 100% herbicide dose significantly decreased total plant biomass to 27.1 g plant^{-1} compared with control (38.6 g plant^{-1}) and spraying with 50% dose (34.1 g plant^{-1}). The difference between control plants and plants sprayed with 50% dose was not significant. *Cirsium arvense* sprayed at 3–7 leaves had significantly lower total weight (25.6–33.8 g plant^{-1}) compared with plants sprayed at eight leaves (38.8 g plant^{-1}).

When spraying at different development stages of *C. arvense*, significant differences in total biomass were observed in the treatment combination 100% dose + crop. Herbicide treatment at three- and four-leaf stage significantly reduced plant weight to 4.2 and 3.0 g plant^{-1}, respectively, compared with the control (17.1 g plant^{-1}) (Figure 1(B)). In the other treatment combinations (100% dose + no crop; 50% dose + crop, and 50% dose + no crop), no such effects were observed.

Total plant biomass at spraying and at harvest

When comparing the changes in total plant weight over time for each phenological stage, plant weight did not increase significantly between spraying and harvest when *C. arvense* grew with spring barley and was treated with full (100%) or reduced dose (50%). The lowest plant weights at harvest were found when plants were sprayed at four leaves (100% dose) (Figure 1(B)) and three leaves (50% dose) (5.8 g plant^{-1}). In the other two treatment combinations (100% dose + no crop, and 50% dose + no crop), total plant weight increased significantly over time for all phenological stages. Total plant weight at harvest was on average 52.0–88.2 g plant^{-1}.

Shoot height

Maximum shoot height at harvest

Maximum shoot height at harvest was significantly affected by crop, spraying, dose and phenology (Table 2). *Cirsium arvense* growing together with spring barley had a significant lower shoot height (40.2 cm) compared with plants growing without a crop (53.2 cm). Sprayed plants had a significantly lower height (36.3 cm) compared with unsprayed plants (57.8 cm). *Cirsium arvense* treated with 100% had significantly lower shoot height (30.8 cm) than untreated plants (57.8 cm) and plants treated with 50% dose (42.2 cm). Plants sprayed at three and four leaves had significantly lower shoot heights (25.5 and 21.7 cm, respectively) compared with control plants (57.8 cm) and plants sprayed at 5–8 leaves (35.6–57.7 cm).

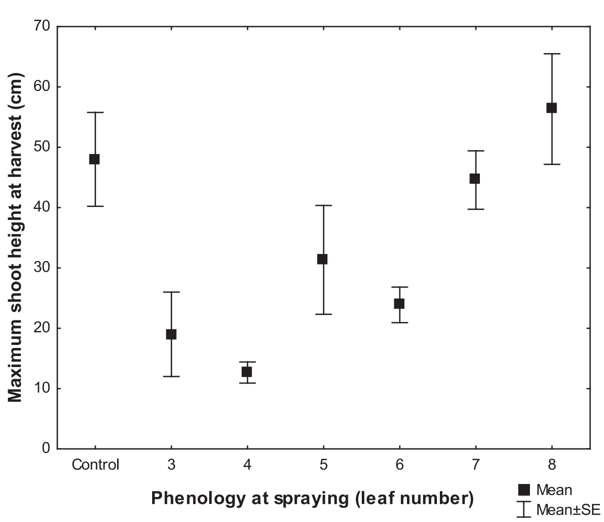

Figure 2. Maximum shoot height of *C. arvense* (cm) (mean ± SE) on raw data at harvest (■) grouped by phenology at spraying and categorised by dose (100%) with the presence of a crop (spring barley). Control at harvest was used to do a pairwise comparison with each of the phenological stages of 3–8 leaves at harvest.

Cirsium arvense plants grown together with a crop and sprayed with a full dose (100%) at the four-leaf stage showed a significant decrease in maximum shoot height compared with the control, maximum heights being 12.8 and 48.5 cm, respectively (Figure 2). Plants growing without spring barley and sprayed with a full dose (100%) at three- and four-leaf stages had significantly lower shoot heights at final harvest (18.6 and 12.3 cm, respectively) compared with the control (69.8 cm). *Cirsium arvense* growing with spring barley and sprayed with 50% dose had significant lower shoot height at harvest when sprayed at leaf stage 4 (24.8 cm) compared with the unsprayed control (48.5 cm). No significant differences in maximum shoot height at final harvest were observed in the treatment where *C. arvense* plants were growing without a crop and sprayed with a reduced dose (50%).

Maximum shoot heights at spraying and at harvest

When assessing height growth after treatment, no significant increases in shoot height were found over time when *C. arvense* was treated with a full dose (100%). The lowest shoot heights at harvest were observed when plants were sprayed at (i) four leaves and grown with spring barley (Figure 2), and (ii) three and four leaves growing without a crop (21.2 and 15.5 cm), respectively. Similar results were obtained when *C. arvense* was sprayed with a 50% dose and grown with spring barley. The lowest shoot heights at harvest (28.4 and 27.5 cm) using this treatment were observed when plants were sprayed at three- and four-leaf stages, respectively. When plants were sprayed with 50% dose and growing without a crop, a significant increase in height was observed at earlier leaf stages (three-, four- and five-leaf stages). At these stages, shoot heights at spraying were 8.6, 10.5 and 14.5 cm, and at harvest 43.3, 39.7 and 52.7 cm, respectively. No significant increase in shoot height over time was found when spraying was performed at leaf stages 6–8 (38.5–58.9 cm).

Relations between median and maximum C. arvense shoot heights

A significant positive correlation was found between median and maximum shoot heights in field populations of *C. arvense* grown with spring barley at Uppsala, Sweden (Figure 3). At a maximum shoot height of 12.8 cm (i.e. maximum *C. arvense* shoot height at a four-leaf stage in the bucket experiment), the median height was about 5.6 cm.

Phenology

Average phenology at harvest

At harvest, an average number of leaves shoot^{-1} was significantly affected by dose (Table 2). *Cirsium arvense* treated with 100% dose had a significantly fewer number of leaves (6.7 leaves shoot^{-1}) compared with the control plants (10.8 leaves shoot^{-1}) and plants treated with 50% dose (10.1 leaves shoot^{-1}).

Cirsium arvense growing with spring barley and sprayed with 100% dose at the three-, four- and the seven-leaf stage had significantly fewer leaves compared with untreated plants (Figure 4). Untreated plants had 12.6 leaves shoot^{-1} while sprayed plants had 5.2 leaves shoot^{-1} (three-leaf stage), 4.6 leaves shoot^{-1} (four-leaf stage) and 5.5 leaves shoot^{-1} (seven-leaf stage), respectively. No significant differences were observed in a number of leaves shoot^{-1} between control plants and plants treated with 100% dose (without crop) or 50% dose (with and without crop).

Average phenology at spraying and at harvest

When assessing leaf production over time, no significant increase in leaf number was found when plants were treated with a full dose (100%) for either of the developmental stages. The lowest leaf production was observed when plants were sprayed at three- and four-leaf stage and grew together with spring barley (Figure 4). When *C. arvense* was sprayed with 100% and grown without a crop, the lowest leaf number at harvest (4.1 leaves shoot^{-1}) was observed when plants were sprayed at the five-leaf stage. Similar results were obtained when *C. arvense* was sprayed with 50% dose. The lowest leaf numbers at harvest were observed when plants were sprayed at three-leaf stage (4.0 leaves shoot^{-1}, with crop) and six-leaf stage (without crop, 6.9 leaves shoot^{-1}).

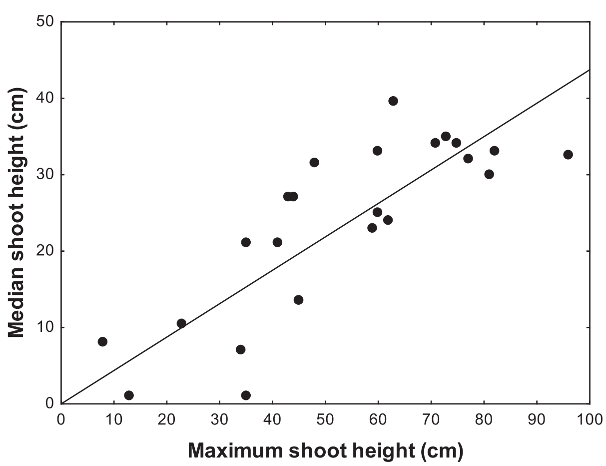

Figure 3. Relation between maximum and median shoot heights of *C. arvense* in a field experiment in Uppsala ($r^2 = 0.66$, $P = .0000$).

Discussion

The major objective of this study was to assess the effects of herbicide dose, the timing of herbicide application, and crop presence on growth and development of *C. arvense*. We wanted to evaluate whether a reduced herbicide dose would give the same control effect as the recommended dose, and if spraying near CP would control *C. arvense* most efficiently. We also investigated whether the presence of a crop would increase the control effects of herbicide treatment. While the use of a pot experiment generates conditions which may differ substantially from field situations, it gave control over the main factors studied and also allowed for a reliable below-ground biomass retrieval, which is difficult to obtain in field situations.

To increase the chances for successful weed control, reduced herbicide rates usually need to be combined with other management methods, such as competitive crops or soil cultivations (Boström & Fogelfors 1999; Zhang et al. 2000; Blackshaw et al. 2006). Our hypothesis, that a reduced herbicide dose would give the same control effect as the recommended dose on growth and development of *C. arvense*, was, however, not supported by the results (Table 2; Figures 1(A) and 2(A)). Even when the herbicide application was combined with a competitive crop (spring barley), the effect of reduced dose was significantly lower than compared

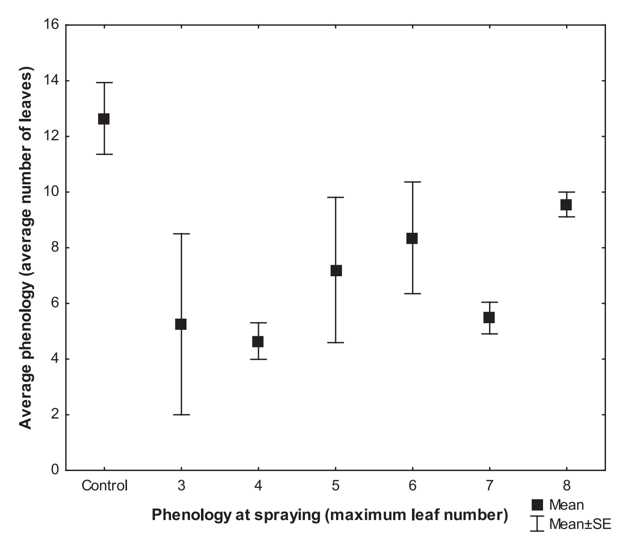

Figure 4. Average phenology of *C. arvense* (no leaves shoot^{-1}) (mean ± SE) on raw data at harvest (■) grouped by phenology at spraying and categorised by dose (100% with spring barley). Control was used to do a pairwise comparison with each of the phenological stages 3–8 leaves at harvest.

with the effect of the recommended dose. This is in accordance with results from a study of perennial weeds (*C. arvense* and *S. arvensis* L.) where reduced herbicide doses of MCPA gave an increase in above-ground biomass as compared with recommended dose (Fogelfors & Lundkvist 2008). A reduction in herbicide dose may give sufficient control effects on weed communities dominated by annual species. Sufficient weed control effects have also been obtained when spraying with reduced herbicide doses against annual weeds in competitive cereal crops (Salonen 1992; Lundkvist 1997). Also, Boström and Fogelfors (1999) stated that it possible to maintain the weed flora on acceptable levels by using 50% lower doses than normally recommended together with appropriate tillage strategies. However, the use of reduced herbicide dosages is associated with the evolution of herbicide resistance (Manalil et al. 2011; Yu et al. 2017) and thus may lead to a decreased possibility to control weeds in the long term.

The effect of a control method is strongly dependent on targeting *C. arvense* at its most vulnerable developmental stage. When using herbicides, *C. arvense* needs to have developed a sufficient number of shoots and leaf area to be able to absorb and translocate the active ingredient down to the root system. In Sweden, spraying is recommended when the major part of the shoots has reached a height of 10–20 cm (Widén & Johansson 2015, 2016; Jordbruksverket 2017a, 2017b) while mechanical control usually is endorsed at a specific developmental stage (CP). At CP, the below-ground structure of *C. arvense* has reached its minimum weight and the plant is considered to be most vulnerable for disturbance (Dock Gustavsson 1997; Håkansson 2003; Verwijst et al. 2018). In this study, we wanted to test whether there was a connection between CP and the optimal effect of chemical control. Therefore, we hypothesised that herbicide treatment close to the three-leaf stage would give the best control effect. We found support for the hypothesis since the strongest control effect on *C. arvense* was obtained when the largest shoot had reached the three- to four-leaf stage (Figure 1). At the four-leaf stage, maximum shoot height was about 13 cm (Figure 2) while the median height was 6 cm (Figure 3). The plants had the same maximum height and number of leaves at both spraying and harvest indicating that growth and development stopped after herbicide application (Figures 3 and 4). Our results differ slightly from current recommendations, i.e. to spray a bit later when the major parts of the shoots have at height of 10–20 cm (Widén & Johansson 2015, 2016; Jordbruksverket 2017a, 2017b). They are, though, in accordance with Tworkoski (1992) who observed that more photoassimilates in *C. arvense* were moved to roots at elongation stage compared with bud and flowering stages and argued that herbicide translocation would be most efficient at these developmental stages. Also smaller plants are more susceptible to herbicide treatment as compared with larger plants (Håkansson 2003). At earlier developmental stages, the *C. arvense* leaves also have a lower lipid content (Hodgson 1973) which might increase the absorption and translocation of the herbicide.

We hypothesised that the presence of a crop would increase the control effect from the herbicide treatments. The rationale was that *C. arvense* is susceptible to reduced light from the shading and competition of neighbouring plants in crop stands and grassland (Bakker 1960; Edwards et al. 2000). The plants produce fewer shoots with less flowers as compared with plants growing in an unshaded environment (Bakker 1960). When growing together with taller or more rapidly growing crop stands like cereals, growth and development of *C. arvense* is therefore usually strongly reduced (Tiley 2010). The hypothesis was supported since the presence of spring barley significantly reduced biomass, shoot height and leaf production of *C. arvense* regardless of herbicide dose (Table 2). Our findings are consistent with for example Christensen (1994) who observed a significant interaction between the competitive ability of spring barley and herbicide performance. When spring barley was treated with 50% and 100% of the recommended dose of MCPA + dichlorprop, the biomass of annual weeds was reduced with 90–100% compared with the untreated control. Similar results were found in a field study by Salonen (1992), where spraying with MCPA/mecoprop in spring barley decreased weed biomass with 75–95%.

We conclude that growth and development of *C. arvense* were most efficiently suppressed when spraying with recommended dose was done when the largest shoot had developed four leaves. Herbicide dose interacted with crop presence, especially at early phenological stages with a low leaf area of *C. arvense*. Crop leaf abundance and relative small leaf area of *C. arvense* likely prevented the herbicide to reach the weed plants sufficiently. At this developmental stage, the shoot population had a maximum shoot height of about 13 cm and a median shoot height of 6 cm. This indicates that herbicide spraying might be performed earlier than previously recommended (when most shoots have reached a height of 10–20 cm). However, to evaluate these results, more studies in both controlled environment as well as in field conditions will be needed.

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