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Regeneration of Canada Thistle (*Cirsium arvense*) from Intact Roots and Root Fragments at Different Soil Depths

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In the present field study, the capability of Canada thistle to develop shoots from intact roots and root fragments at different soil depths was studied. The experiments were performed on four sites with high-density Canada thistle, with three or four replications per treatment. At each site, the soil in the plots was removed layer by layer (to 30 or 40 cm, depending on the site), within a 1 by 1-m quadrat, and spread out on a plastic sheet. All roots and other plant parts were removed, and the soil was either replaced without any root material (two sites), or the roots of the thistles were cut into 10-cm-long fragments and replaced into the source holes (two sites). The measured variables were shoot number and biomass. The number of shoots of Canada thistle decreased with increasing depth ($P < 0.001$) and increased with time. Additionally, the two factors interacted ($P < 0.001$) such that shoot development was slower from greater depths. Roots from $\leq$ 20 cm depth produced higher biomasses than did roots from below 20 cm depth. Replacement of root fragments did not affect the amount of biomass produced. It was concluded that the intact root system contributed considerably more to the total biomass produced by Canada thistle than did the root fragments in the upper soil layers.

Nomenclature: Canada thistle, *Cirsium arvense* (L.) Scop.
Key words: Intact root system, shoot biomass.

Canada thistle and other creeping perennial weeds are of major concern in plant production, particularly in organically grown cereal crop rotations (e.g., Bacher et al., 1997; Cormack, 1999; Salonen et al., 2001). To optimize weed control, understanding the life cycle and growth strategy of specific weeds species is crucial. Soil cultivations intended to control perennial weeds should mainly be focused on reducing the storage of food reserves needed for regrowth (Håkansson 2003).

The roots of Canada thistle may reach a depth of more than 2 m, but the greatest root bud activity occurs above 40 cm (Nadeau and Van den Born 1989). However, if root fragments from different soil levels are planted at the same depth, the shoot production per meter of root is found to be consistent, independent of the original depth from which the roots were excavated (Nadeau and Van den Born 1989). After soil tillage, undisturbed roots of Canada thistle are present below the working depth of the tillage equipment, whereas root fragments of various sizes are distributed throughout the tilled soil profile. The production of new shoots from these fragments may be expected to increase with deep plowing to, e.g., 25 cm, as the fragments are brought higher up in the profile and, therefore, require less energy for shoot production. Studies have, however, shown that deep plowing, e.g., to 25 cm, significantly improves the control of Canada thistle compared with more shallow (15 cm) plowing (Brandsæter et al. 2011). These results may indicate that root fragments play a restricted role for further growth compared with the undisturbed root system. As the aim of the management of perennial weeds is to reduce the food reserves in the storage organs, it is necessary to gain insight into the importance of root fragments as well as that of the undisturbed root system. A number of studies have focused on the regeneration capacity of Canada thistle root fragments of different length and at different soil depths (e.g., Dock-Gustavsson 1997; Sciegienka et al. 2011; Thomsen et al. 2011). The number of shoots and biomass production have been found to be positively correlated with the length of the root fragments (Dock-Gustavsson 1997; Thomsen et al. 2011) and negatively correlated with burial depth (Dock-Gustavsson 1997; Sciegienka et al. 2011; Thomsen et al. 2011). Dock-Gustavsson (1997) also found that the number of leaves per shoot, at the level of minimum regenerative capacity of the root fragments, was negatively correlated with the burial depth. However, little is known about the regeneration of Canada thistle from the intact root system. The present experiments address the following two hypotheses: (1) shoot number and aboveground biomass of Canada thistle change significantly with the depth of the root system, and (2) the intact root system makes a significantly higher contribution to regrowth, measured as the number of shoots and biomass production, compared with that of root fragments.

Materials and Methods

The study included two experimental series, I and II, with two trials in each, carried out at three different sites. The trials in series I were designated 2005-A and 2005-B, and the trials in series II were designated 2006 and 2011.

Site Descriptions. *Experiment I*. Experiment I was carried out in 2005: 2005-A at a site located 5 m above sea level [asl] and 2005-B 200 m asl, both on imperfectly drained loam soil. During the two seasons before the experimental period, both fields had been cultivated with a green manure crop in an organic crop rotation. The fields were plowed for the last time in autumn 2002, at 20 to 25 cm depth. The aim of these experiments was to investigate the capacity of the intact root system of Canada thistle to develop shoots from different soil depths.

Experiment II. Experiment II was performed in 2006 and 2011 at a site 90 m asl on a sandy loam soil. Previously, the field had been farmed conventionally for a number of years,

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Table 1. Times of establishment of the trials, observations, and harvesting.

	2005-A	2005-B	2006	2011
Date of establishment	April 26	May 3	July 19–25	May 14–16
Observations, d after establishment	30	34	19	15
	41	47	30	31
	55	59	53	49
	90	91	84	90
	111	115		
Harvest date	August 17	August 26	October 15	August 25

mainly with spring cereal crops, and plowed to 20 to 25 cm depth in late October or early November. The aim of these experiments was to investigate and compare the capacity of the intact root system and that of root fragments to produce shoots from different soil depths.

Trial Description and Assessments. All experiments were arranged in complete randomized blocks with three (2005-B, 2006, and 2011) or four replications (2005-A).

Experiment I. An experimental area densely populated with Canada thistle was chosen. The trials were established in the spring of 2005, with 2005-A on April 26 and 2005-B on May 3, and were harvested on August 17 and August 26, respectively (see Table 1). The regeneration of the weed from intact root systems located below five different soil depths (0, 10, 20, 30, and 40 cm) was evaluated. In each plot, the soil was dug out down to the given depths within a 1 by 1-m quadrat and spread out on a plastic sheet, leaving roots below the given depths undisturbed. All roots and other plant parts of the excavated soil were removed. Then the root-free soil was replaced into the source hole and packed down to its original level (see Table 2). In the control plot (0 cm depth), the thistle plants were mowed (stubble height, 2 to 3 cm), and the soil was left untreated. Throughout the season, the shoots were counted. In the autumn, the aboveground biomass of Canada thistle was harvested and dried at 70 C for 72 h, and the dry weight was recorded.

Experiment II. These two experiments were started on July 19 to July 25, 2006, and May 14 to May 16, 2011, and were harvested on October 15, 2011, and August 25, 2011, respectively (see Table 1). The soil was dug out to depths of 0, 15, and 30 cm and handled as in Experiment I, but with two different treatments of the soil from 15 and 30 cm depths: either (1) all roots and other belowground plant parts of Canada thistle were removed from the excavated soil, or (2) all belowground plant parts were replaced together with the soil (see Table 2). In 2006, the roots were cut into 10 cm lengths before reburial. To avoid drying of the root fragments during the time between when they were excavated from the soil and cut into fragments and when they were placed back into the hole, they were kept on wet paper in a cooler. In plots with 15 cm digging depth, half of the root pieces were placed at 5 cm depth and the other half at 15 cm depth. In plots with 30 cm digging depth, one-third of the roots were placed at each of the depths of 5, 15, and 30 cm. In 2011, the roots were cut into 10-cm-long fragments but were kept in close contact with the soil and within a short time were randomly distributed within the returned soil. The number of shoots was counted during the growing season. At the end of the experimental period, the aboveground biomass of Canada thistle was harvested and dried at 70 C for 72 h, and the dry weight was determined.

The distance between the plots in each block was 1 m. To prevent Canada thistle in the surrounding area from growing into the experimental plots later in the season, that area was frequently mown to 2 to 4 cm stubble height throughout the summer. Registrations were concentrated to the inner 80 by 80 cm of the 1 by 1-m quadrats. This was done to exclude shoots originating from roots bordering up to the experimental plots.

Table 2. Schematic presentation of the treatments applied in the four trials.

Digging depth	Root fragments removed (−) or replaced (+)
cm	
2005-A/2005-B	
0	
10	−
20	−
30	−
40	−
2006/2011	
0	
15	+
15	−
30	+
30	−

Statistical Analyses. The statistical evaluation was performed using the statistical software SAS (SAS Version 9.2, SAS Institute, Cary, NC) and MINITAB (MINITAB 16, Minitab Inc., State College, PA). The assessments of the number of shoots over time were made on the same plots and were, therefore, modeled using a linear mixed model, taking care of the repeated-measurements structure. The main effects of time and digging depth and their interaction were tested for significant effects. Effects were considered to be significant when $P \leq 0.05$. In cases where the data on shoot number (y) did not follow a normal distribution with a constant variance, we used the $\log_{10}(y + 1)$ as a transformed response variable. Before being presented in tables and figures, the data were detransformed. The initial number of Canada thistle plants was used as a covariate; where it did not contribute significantly to the model ($P > 0.10$), that covariate was omitted. To model the repeated-measurements structure, different models, with and without a transformed response variable and with different covariance structures, were considered. The final model was chosen based on an appraisement of Akaike's information criterion and residual plots checking normality and constant variance.

Differences in the aboveground biomass (z) were tested using ordinary ANOVA models and appropriate F tests (MINITAB 2011). In cases where the data did not follow a

Table 3. ANOVA table with P values for the analysis of weed responses.

			P value			
			No. of shoots	Biomass	No. of shoots	Biomass
Experiment	Fixed effects	df	2005-A		2005-B	
I	Time (T)	4	< 0.001[a]	—	< 0.001	—
	Depth (D)	4	< 0.001[a]	0.025	< 0.001	0.021[a]
	T × D	16	< 0.001[a]	—	< 0.001	—
			2006		2011	
II	Time (T)	2	< 0.001	—	< 0.001[a]	—
	Depth[b] (D)	4	< 0.001	< 0.001[a]	< 0.001[a]	0.001
	T × D	8	< 0.001	—	< 0.001[a]	—

[a] Log_{10}-transformed values (y + 1).
[b] Including removal or replacement of root fragments.

normal distribution with constant variance, we used the $\log_{10}$ (z + 1) as a transformed response variable.

Tukey's pairwise comparison test with 95% confidence level was used to test for significant differences between individual treatments with respect to our response variables (the number of shoots y and the aboveground biomass z). The comparison of aboveground biomass within the groups ≤ 20 cm digging depth and > 20 cm digging depth were tested using a t test (2005-A) or a Mann-Whitney rank sum test (2005-B).

Interpretation of main effects and main-effect comparisons were only done in cases where no interactions were found. Otherwise, the interactions were described and interpreted.

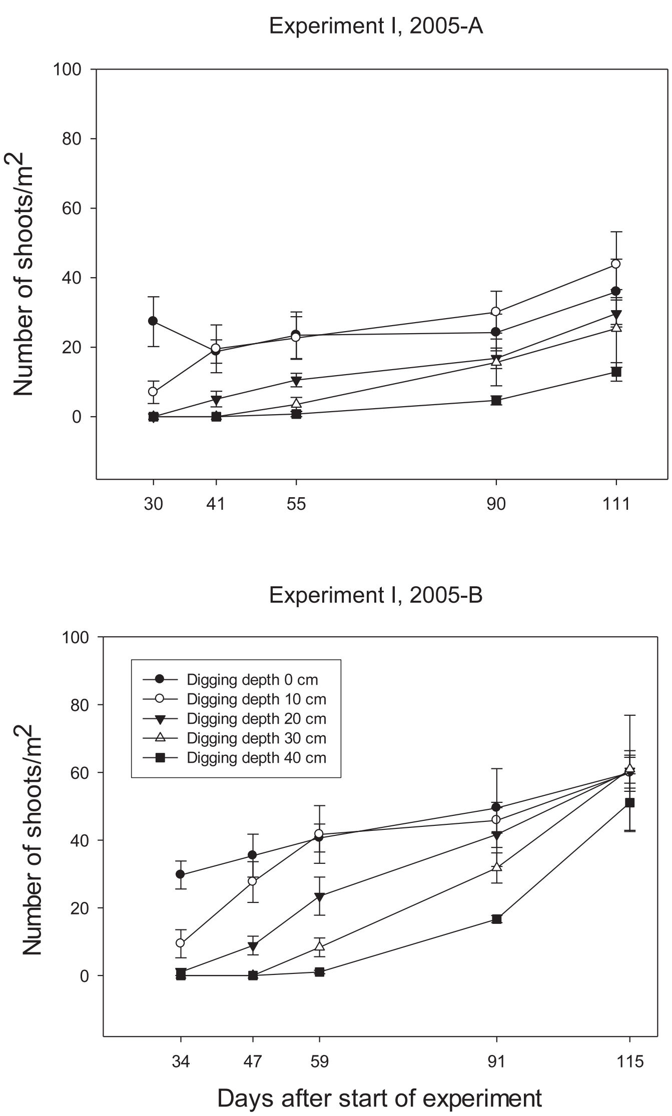

Figure 1. Development in the number of shoots during the experimental period in experiment I as affected by digging depth (vertical bars: ± SE).

Results

Experiment I. *Number of Shoots*. At both sites, the number of Canada thistle shoots increased with time and decreased with digging depth, and interactions between depth and time were detected (Table 3; Figure 1). Shoot development was slower at greater digging depth, and shoot development varied with time for the different digging depths (Figure 1). For a digging depth of 40 cm, aboveground shoots were visible after 55 or 59 d for sites 2005-A and 2005-B, respectively, and continued to increase after that. For a digging depth of 30 cm the corresponding dates were 41 or 47 d after establishment, respectively. The production of shoots from a digging depth of 10 and 20 cm started at the first observation date (30 or 34 d, respectively). After approximately 55 or 59 d, a stagnation in shoot development was observed, which was more pronounced for the digging depth of 10 cm than it was for the 20-cm digging depth and was also more pronounced at site 2005-A, compared with site 2005-B and lasted until 90 or 91 d after establishment of experiment, when shoot production again increased. The control treatment had, in general, slower shoot development. However, a continuous increase in shoot number with time was found at all depths.

Aboveground Biomass. The biomass of Canada thistle was affected by the digging depth at both 2005-A and 2005-B sites (Table 3). At 2005-A, removal of the roots down to10 cm led to higher biomass than root removal down to 40 cm. At 2005-B, the biomass produced in the control treatment (0 cm) or after removal of the roots down to 10 cm depths was higher than when the roots were removed down to 40 cm depth (Figure 2).

By grouping the data, the biomass production was found to be higher on shoots emerging from digging depths ≤ 20 cm than that from greater digging depths (Table 4).

Experiment II. *Number of Shoots 2006*. The number of shoots was reduced with increased digging depth, and interaction between time and digging depth was detected (Table 3; Figure 3). The sprouting pattern within each

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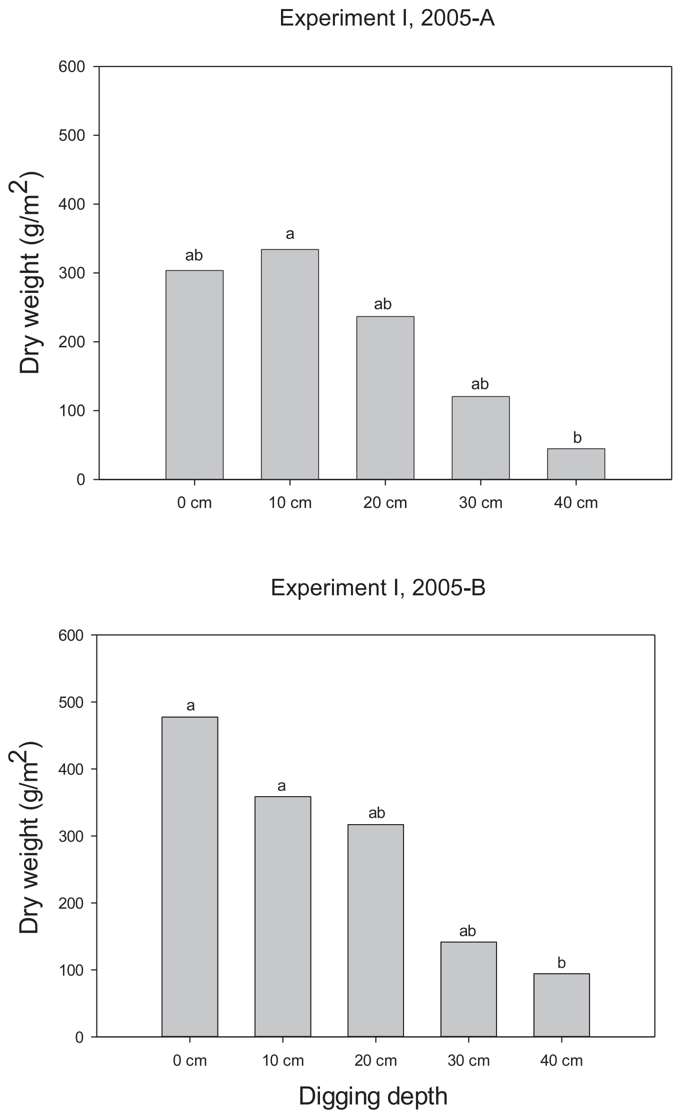

Figure 2. Dry weight of aboveground biomass in experiment I as affected by digging depth. At each site, the soil was excavated to the depths given on the x-axis, $n = 4$ in 2005-A, and $n = 3$ in 2005-B (different letters indicate significant differences between treatments).

digging depths, with and without root fragments, showed very similar development during the experimental period. Shoot development was slower from greater digging depth, and shoot development varied with time for the different digging depths (Figure 3). The number of shoots increased for both digging depths of 15 and 30 cm with or without the removal of shoots, from the first observation until 53 d after establishment, where the number of shoots was reduced. For the control treatment, there was a continuous decrease in the number of shoots after establishment of the experiment. Removal or replacement of root fragments did not influence the shoot number within each digging depth.

Number of Shoots 2011. The combination of digging depth, with or without root fragments, and time affected the number of shoots, and an interaction between time and digging depth was found (Table 3). The number of shoots continued to increase throughout the experimental period (Figure 3). For the digging depth of 30 cm, with or without removal of shoots, the number of shoots increased 31 d after establishment. The 30-cm treatment and replacement of root fragments seemed to increase the number of shoots more rapidly than did the 30-cm digging depth where the root fragments were removed.

Table 4. Aboveground biomass (mean of the observations) of Canada thistle in relation to the depths of the intact root system categorized into two groups based on digging depths of 0, 10, 20, 30, and 40 cm.

Sites	Aboveground biomass mean — Depth of intact root system: ≤ 20 cm	> 20 cm	P
	——— g ———		
2005-A	291.4 ($n = 12$)	82.5 ($n = 8$)	< 0.001
2005-B	384.4 ($n = 9$)	117.9 ($n = 6$)	0.002

Aboveground Biomass 2006. The control treatment and digging depth to15 cm, with or without replacement of the root fragments, resulted in greater biomasses than did any treatment with a 30-cm digging depth. No difference in biomass was found between removing and replacing the belowground plant parts within each digging depth (Figure 4).

Aboveground Biomass 2011. The control treatment produced a greater biomass than did the digging depths of 15 cm, with roots removed, and 30 cm, both with and without removal of the roots. Digging to 15 cm and replacing the roots yielded a higher biomass than did digging to 30 cm, with or without replacement of the roots. Within each digging depth, neither removal nor replacement of the root fragments affected the aboveground biomass (Figure 4).

Discussion

The results on Canada thistle shoot production in these experiments corresponded well with those of field studies, which showed that the number of shoots decreased significantly with increased plowing depth (e.g., Brandsæter et al. 2011; Gruber and Claupein 2009).

The depth of intact root influences shoot and biomass production, but the production from roots at greater depth was found to be vigorous. Shoots developing from root fragments, e.g., after soil tillage, appear to make a limited contribution to the total number of shoots or biomass produced when, as in the present study, the fragments are located above the intact root system. The lack of effect from removal or replacement of root fragments in the 2006 experiment may have been a consequence of the late trial establishment in that year (July). The roots may not have recovered fully after the exhaustion caused by the development of shoots in spring, as noted by Fykse (1977), and aboveground shoot production ceased before the end of the experiment. However, in the 2011 experiment, which was established in May, no difference was observed between the removal and replacement treatments either. On the other hand, the production of biomass was, because the longer growing period, in contrast to 2006, considerably higher, and a continuous increase in the number of shoots was observed in in the 2011 experiment. The overall explanation may, therefore, be that the undisturbed part of the root system,

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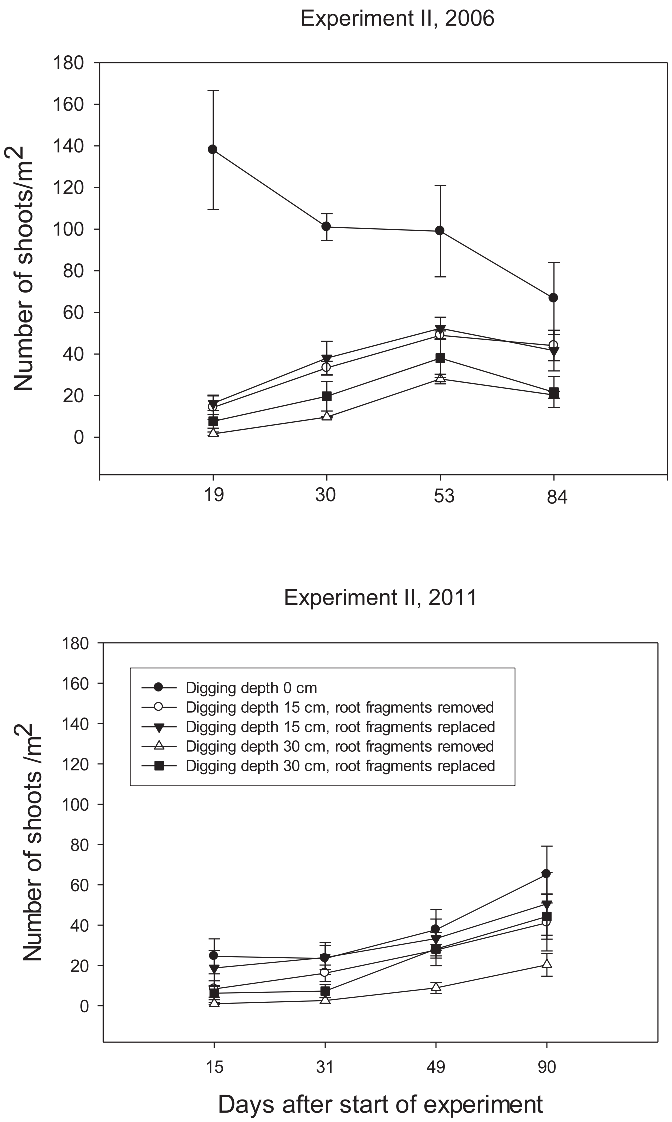

Figure 3. Development in the number of shoots during the experimental period in experiment II as affected by digging depth (vertical bars: ± SE).

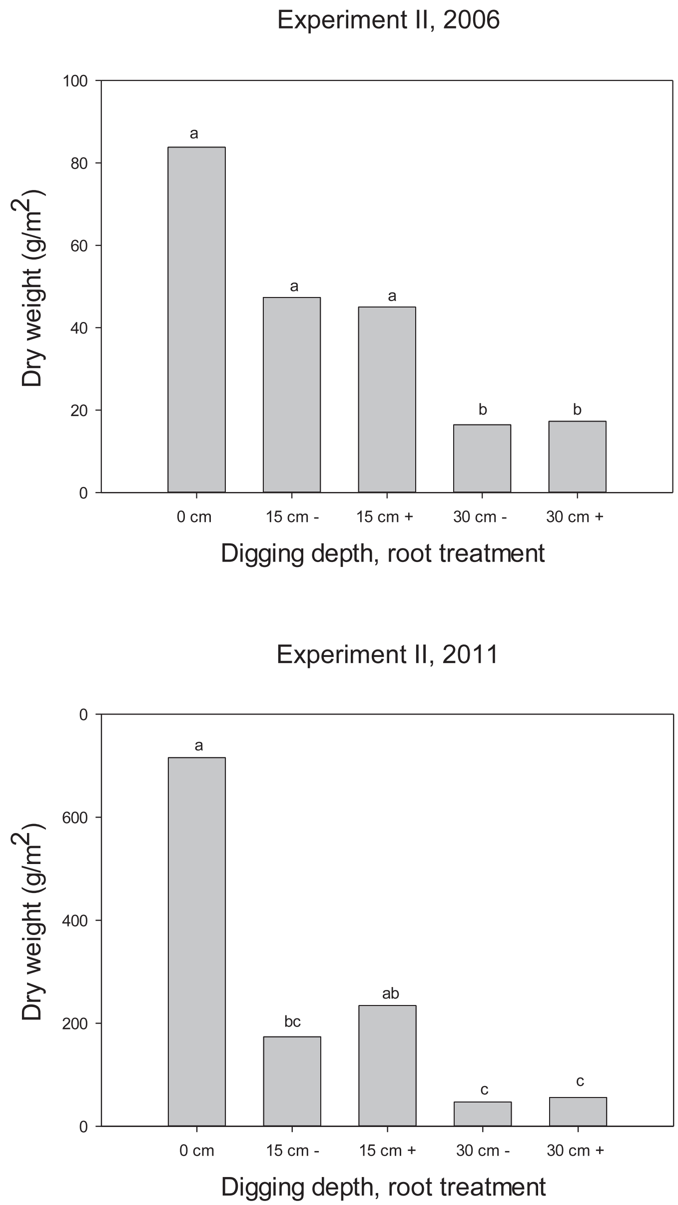

Figure 4. Dry weight of aboveground biomass in experiment II as affected by digging depth (different letters indicate significant differences between treatments). At each site, the soil was excavated to the depths given on the x-axis: Abbreviations: −, roots of Canada thistle that were removed from the soil; +, roots were replaced ($n = 3$ in both 2006 and 2011; note the different scaling of the y-axis in 2006 and 2011).

located below the upper tilled volume of the soil, possessed a high regenerative capacity, which may have overshadowed the shoot production from the root fragments. If, however, the root fragments are spread to new areas, a number of studies have shown that they may easily establish and represent a high risk of infestation (e.g., Thomsen et al. 2011). High diversity in genotypes between neighboring patches has nevertheless led to the conclusion that dispersal of root fragments via soil cultivation is of minor importance compared with seed dispersal (Hettwer and Gerowitt 2004).

The stagnation in the number of shoots in experiment I for digging depths of 10 and 20 cm, as well as for the control treatment in 2005-A, may have been similar to the sprouting stagnation observed by Brandsæter et al. (2012). In that study, however, there were no observations on a new flush in shoot production later in the season. The stagnation seemed more pronounced for a digging depth of 10 cm than it was for a 20-cm digging depth. The reason for that could be related to the time of minimum regenerative capacity and following shoot independency, as documented by Nkurunziza and Streibig (2011), causing a possibly delay in new shoot development. In experiment II, the same stagnation was not found in the 2011 experiment, whereas, in 2006, there was a clear reduction in shoot number after 53 d, which was likely due to the late establishment of this experiment in late July, when the other three experiments were established between late April and the middle of May.

The excavation and replacement of root fragments may have lowered the soil moisture content, thereby reducing the ability of the root fragments to sprout (Niederstrasser and Gerowitt 2008). However, the same results were found in both years, and in both years, the summer rainfall was about or above average (95.3% and 193%, respectively) (Norwegian Meteorological Institute yr.no, 2012). Moreover, the size of the fragments used in the present experiment should have been sufficient for shoot production and establishment

(Dock-Gustavsson 1997; Nkurunziza and Streibig 2011; Thomsen et al. 2011).

In these experiments, we attempted to reduce the encroachment of growth from outside the plots by mowing the surrounding area. When the shoots of Canada thistle are continuously mown, the transport of nutrients to the roots is hampered, and the growth of new regenerative structures may be reduced (Graglia et al. 2006; Gruber and Claupein 2009). New regenerative structures of creeping perennial weeds may not develop before a species-specific stage of development is reached, which, for Canada thistle, is the four- to seven-leaf stage (Håkansson 2003). Canada thistle displays high shooting capacity during May, whereas, later on, the number of shoots is more or less stable (Brandsæter et al. 2012). When the present experiments were established, roots from plants bordering the plots may have already grown into the plots. However, the regular mowing is likely to have strongly reduced production of new roots and shoots later in the season (Håkansson 2003).

The results of the present experiments are important to understanding the regenerative capacity of the deeper, undisturbed root system of Canada thistle and the threat that weed poses for infestation of cropland. Studies concentrated on root fragments should, therefore, be interpreted with these results in mind. As a single measure, deep cutting of the root system will delay and reduce the shoot and biomass production more than will shallow treatments. However, the results show that the intact root system is highly vigorous below normal tillage depth. To control Canada thistle by reducing its food reserves, repeated and varied treatments are, therefore, needed, such as timely cuttings either above or below the surface to prevent translocation of assimilates to the belowground plant parts, as well as increasing the competition from other plants during the growing season (Dock-Gustavsson 1997; Graglia et al. 2006; Thomsen et al. 2011). The management of Canada thistle should be an integrated part of mechanical weed control and other cultural practices and will probably only be successful when preventive measures and cultural weed control are combined (Bàrberi 2002).

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