Drought Tolerance and Perennial Weed Management

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The aim of this study was to investigate the effect of controlled soil water deficits on sprouting and shoot growth of Canada thistle, coltsfoots and quackgrass. A gradient of soil water contents was created by establishing different densities of barley. The plants were harvested 14 days after watering was stopped. On Canada thistle and coltsfoots, relative water content (RWC) in leaves was measured prior to harvest and biomass of all weed shoots were recorded at harvest. In terms of shoot biomass and leaf RWC quackgrass was drought tolerant while coltsfoot was drought sensitive and Canada thistle was between the two. The barley cover crop could have had a competitive effect upon the growth of the weeds; the effect, however, was not detrimental compared to the drought effect, because relationships between initial height and the final height of coltsfoot and Canada thistle were not different among barley densities. The results suggest that the shooting from subterranean parts of broadleaf perennial weeds can to some extent be impeded by reducing soil water availability. However, the use of reduced soil water content can be challenging in fields in humid temperate regions.

Nomenclature: Barley, *Hordeum vulgare* L.; Canada thistle, *Cirsium arvense* (L.) Scop; Quackgrass, *Elytrigia repens* (L.) Nevski; Coltfoots, *Tussilago farfara* L.

Key words: Soil water content, early growth, broadleaf perennial weeds.

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Drought adaptation divides plant species of seasonal environments into two categories: annual species known to be drought avoiding and perennial species that are drought tolerant (Zollinger et al. 2006). For both categories, a range of drought adaptation mechanisms have been recently described (Farooq et al. 2009). Perennial species forestall desiccation, tolerate desiccation, or they combine both strategies. It has been established that modulations of cellular elasticity and osmotic potential, via solutes accumulation, are important mechanisms used to tolerate drought in perennials during dry and hot summers (Hare et al. 1998;Yousfi et al. 2010).

It is only under favorable environmental conditions (e.g. temperature, water availability) that herbaceous perennials resume their vegetative growth, otherwise the above-ground biomass dies back and the biochemical and physiological activity remain at a minimum (growth arrest) until the return of appropriate conditions. Lundmark (2007) and Patton et al. (2007) have shown the association between plant cell water and carbohydrates in propagules of perennials of temperate regions. In weed management, tillage has been traditionally recommended for control of quackgrass (*Elytrigia repens* (L.) Nevski) because of increased rhizome desiccation when exposed on the soil surface. However, owing to humid autumn climate and biotype differences, high variations in sprouting has been demonstrated (Reidy and Swanton 1994; Melander et al. 2008) and this requires additional strategies to destroy rhizomes and rootstocks once exposed to desiccation on the soil surface. In addition, exposure and destruction of rhizomes and rootstocks apply to weed species that can be completely uprooted such as quackgrass. Species with deep root/rhizome systems such as Canada thistle (*Cirsium arvense* (L.) Scop) and coltsfoot *(Tussilago farfara* L.) would escape uprooting. These species make up a considerable problem in conventional as well as in organic farming of the temperate regions (e.g. Andreasen and Stryhn 2008; Hyvönen et al. 2003, Lundkvist et al. 2008; Lukashyk et al. 2008)

Great amount of carbohydrate reserves, mainly fructans, stored in reproductive roots of Canada thistle and rhizomes of coltsfoot (Otzen and Koridon 1970) support shoot emergence when favorable temperature and soil moisture are available. However, among the few studies that have addressed, the early growth of perennials at different levels of soil water content (Gordon et al. 1999*;* Gazanchian et al. 2006; Kawakami et al. 2006); none have focused on herbaceous perennials with deep underground root or rhizome systems and broad leaves. The information on levels of drought tolerance during the establishment period in different types of perennial weeds may help farmers improve control strategies in organic farming systems.

The objective of this study was to compare the drought tolerance of quackgrass, Canada thistle and coltsfoot by measuring shoot production response under a gradient of soil water contents. Since small leaf size can be seen as an adaptation to drought (Pedrol et al. 2000; Kulkarni et al. 2008; Xu et al. 2009), we hypothesize that drought tolerance is higher for quackgrass than the two other broad leaf perennial weeds.

Material and Methods

Plant Material. Uniform and approximately five centimeters long root/rhizome fragments of Canada thistle[1], coltsfoot[2] and quackgrass[3] were planted at 5 cm depth in 4 L pots (15 cm of diameter, 25 cm depth) with sandy soil. The exact length in case of rhizomes of coltsfoot and quackgrass was dictated by the presence of at least one lateral bud at the node of rhizomes to ensure the possibility of shooting.

Experimental Design. An experiment was run twice in greenhouse at the experimental station in Taastrup, University of Copenhagen, Denmark (55 40´10N; 12 18´32E) from July to September 2009 and from March to April 2010. The experimental factors were three weed species and five levels of soil water content in a randomized complete block design with 8 and 10 replicates in the first and second experimental runs, respectively. The five levels of soil water content, for each species, was created by four different cover densities of barley[4] (*Hordeum vulgare* L., SIMBA 08T5 Øko) and a reference without barley giving a total of five densities. The cover densities were obtained by sowing 5, 10, 15 and 20 barley seeds per pot one day after a root or rhizome had been planted. In total, 120 and 150 pots with four liters of sandy soil were used in 2009 and 2010, respectively.

Prior to root and rhizome planting, pots containing soil were watered to field capacity. Thereafter drip irrigation with a fertilizer solution[5] was applied with 25 cm^3 water per pot per day. The drip irrigation continued until 30 % shoots had emerged from roots or rhizomes. At this time watering stopped and soil water content measurements began with an interval of 2 to 3 days and up to harvest. The drought deficit lasted for a period of approximately 14 days in both experimental replications.

Measurements. Measurements of soil water content were done with HydroSense[6] (HydroSense^{TM,} Campbell Scientific Australia Pty. Ldt). At each time, two measurements were taken per pot. These measurements were coupled with growth change assessment by taking the height of weeds, except for coltsfoot that has a compacted stem. At the end of the 2010 experimental run, in Canada thistle and coltsfoot we measured leaf fresh weight (FW), turgid weight, (TW), which is the difference between weight of a newly detached leaf form the drought suffering plant and the leaf after being soaked in water for 4 hours, and dry weight (DW) and the calculated relative water content (RWC=

(FW-DW)/(TW-DW)) (Liu and Stützel 2002). Only broadleaf weeds, Canada thistle and Coltsfoot, were used for RWC to compare their tolerance to water stress.

At the end of the experiment, the weeds were harvested. Aboveground parts were weighed and dried at 70°C until constant weight.

Statistical Analysis. Linear regression was used to analyze the relationship between the soil water content, cover crop densities, shoot biomass and RWC. Subsequently, t-test was used to test differences in regression slopes of soil water content on cover crop density at different measuring time within species.

We used analysis of covariance to assess the relation between final height and initial height of the weed species. First, we tested the interaction between final height (Y) and initial height (x) at different barley densities (i=1..5) (eq. 1);

$$Y = \alpha_i + \beta_i x \quad [1]$$

Subsequently, we tested if the interaction could be ruled out by assuming similar slope but different intercept (eq. 2)

$$Y = \alpha_i + \beta x \quad [2]$$

And finally we tested if the intercept could be assumed to be the same for all barley densities,

$$Y = \alpha + \beta x \quad [3]$$

All data analyses were done with the open source program **R**[7]

Results and Discussion

Different densities of barley created a gradient of soil water content as expected. Soil water content decreased with increasing barley density for all species and time. The regression slopes of soil water content in percent on barley density within species (Table 1) were in most instances not significant from each other at the $P>0.05$ level. The only differences were the regression slopes at day zero for coltsfoot and quackgrass after the irrigation was stopped (Table 1). This consistency of the slopes of the regression of soil water content on barley density in Table 1 allowed us to use mean soil water content for comparisons of biomass and relative water content (Figures 1 and 2). Table 1 also gives the slopes of the regression of biomass on soil water content (Figure 1).

The results showed that the responses of shoot biomass to soil water content were different among weed species. Coltsfoot (Figure 1A and D) had the larges regression slope and was thus more sensitive to drought stress than the other species. There was a positive significant change of the shoot biomass as soil water content increased for coltsfoot in both experiments (Figure 1A and

D), but this was only observed once for Canada thistle in the 2009 run (Table 1B and E). There was no significant relationship in any of the experiments for quackgrass (Table 1C and F).

The differences found in shoot biomass of coltsfoot and Canada thistle in relation to soil water contents also were reflected in the data of relative water content of leaves on soil water content in Figure 2. The difference between the two slopes, however were only significant on the $P = 6\%$ level. At higher soil water contents, coltsfoot had higher relative water content than did Canada thistle. The steeper slope for coltsfoot than for Canada thistle indicated that the former species is more susceptible to soil water deficit. These results support previous observations on the effect of soil water differences and germination or vegetative reproduction (Håkansson 2003a; Håkansson 2003b).

Previous research on soil water management in relation to root dry weight, leaf area and the number of inflorescences was done on Canada thistle (Zimdahl et al. 1991) and the effect of moisture in interaction with Glyphosate on the same species (Tworkoski et al. 1998). These studies, however, were conducted at a later growth stage compared to the sprouting and establishment, which was used in our study. In addition, no comparative studies have been conducted so far, where we can see how various broadleaf weeds behave among themselves and in relation to quackgrass (Figure 1). It means that controlled soil water deficits may be used as a tool to reduce the early establishment of perennial weeds. In a field perspective, crop density can to some extent be used to reduce the infestation of perennial weeds as already pointed out elsewhere by increasing the competition ability of the crop to take up water, nutrition and utilize sunlight (e.g. Weiner et al. 2001; Kristensen et al. 2008).

The analysis of covariance showed, by means of sequential tests, that the relationship between the final height and the initial height in Figure 3 was independent of barley density in the pots. In other words, the final height was not interacting with the barley density during the 14 days of drought. Consequently, the regression in Figure 1 for biomass on soil water content was mostly affected by the sheer soil water content and not the competitive effect of the barley density. Of course this does not necessarily mean that barley did not have an effect on the growth of the weeds, apart from drying out the soil, but apparently the period of the drought was not enough to show clear cut competition effects. We cannot rule out, however, some confounded effect between barley density competition and draught. That said the duration of the drought period was rather short, only 14 days.

We recognize the difficulty in using water stress as a management strategy in wet and humid temperate regions. But there are indications that water stress can be used as a method to control perennial weeds in arid zones with dry and hot summers, when it can be economically justified (Kjelgren et al. 2009). With some extrapolations, these results might also open up new perspectives especially in arid zones. For instance, tillage followed by irrigation, during dry and hot summers, would deplete carbohydrate storages and reduce infestation at the return of rain. However, a study on bulbs which contain both fructan and starch found that the degree of polymerization of fructan increased after drought (Orthen 2001). More research is needed to elucidate the relationship between the increased degree of polymerization of fructan, the effect of drought and the amount of energy available in the roots and rhizomes for sprouting and establishment.

Sources of Materials

[1, 2, 3 and 4], Roots of *Cirsium arvense*, rhizomes of *Tussilago farfara* and *Elytrigia repens* and seeds of *Hordeum vulgare* from the experimental station of the University of Copenhagen, 2630 Taastrup, Denmark. The planting material of *Elytrigia repens* used at the second experiment was obtained from Research Centre of Flakkebjerg at the University of Århus, 4200 Slagelse, Denmark.

[5] Pioner NPK Makro 14-3-23 + Mg plus Pioner micro with ion, Brøste A/S, Denmark

[6] HydroSense for measurement of water content (HydroSense^{TM,} Campbell Scientific Australia Pty. Ldt)

[7] The **R** Foundation for Statistical Computing, Version 2.10.1 (2009-12-14). http://www.R-project.org

Acknowledgements

Financial support from the International Centre for Research in Organic Food Systems (ICROFS, Denmark) is acknowledged. We thank Jens Bertelsen, Hanne Hasselager and Britta Garly Henriksen for technical assistance in the greenhouse experiments. We also thank Solvejg K. Mathiassen, from the Research Centre of Flakkebjerg at Århus University, who provided rhizomes of *Elytrigia repens* for the second experiment.

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Table 1. Linear regression slopes of soil water contents (SWC) on Barley densities (BD); and shoot biomass on SWC and of different species. Time denotes days after irrigation stopped.

Relationship	Time Days after stop of watering	*Canada thistle*	*Coltsfoot*	*Quackgrass*
SWC vs. BD	0	-0.40(± 0.075)	-0.19 (± 0.063)$^{\#}$	-0.22 (± 0.071)$^{\#}$
	3	-0.47(±0.079)	-0.33 (±0.067)	-0.27 (±0.065)
	6	-0.40(± 0.044)	-0.35 (± 0.070)	-0.35 (± 0.054)
	9	-0.59(±0.052)	-0.52 (±0.061)$^{\#}$	-0.48 (±0.064)$^{\#}$
	14	-0.43(± 0.051)	-0.34 (± 0.063)	-0.41 (± 0.055)$^{\#}$
Biomass vs SWC	Exp. 2009	0.18 (± 0.069) *	0.46 (± 0.090) ***	0.02 (± 0.016) ^{NS}
(see Figure 1)	Exp. 2010	0.03 (± 0.019) ^{NS}	0.26 (± 0.091) **	-0.02 (± 0.010) ^{NS}

Significance levels: ***: $P<0.001$; **: $P<0.01$; *: $P<0.05$; NS: $P>0.05$

$^{\#}$ Slope within species significantly different from each other.

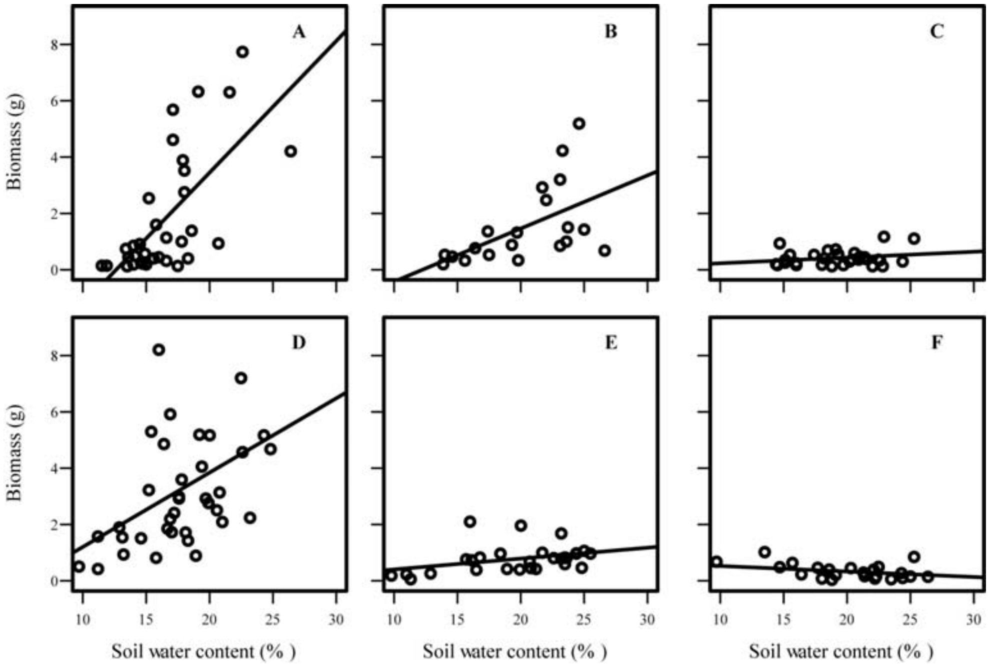

Figure 1: Relationship between biomass of shoots of *coltsfoots (A and D)*, *Canada thistle* (B and E) and *quackgrass* (C and F) and soil water content. The upper graphs represent the first experimental run while the lower graphs are from the second experimental run (Regression slopes in Table 1).

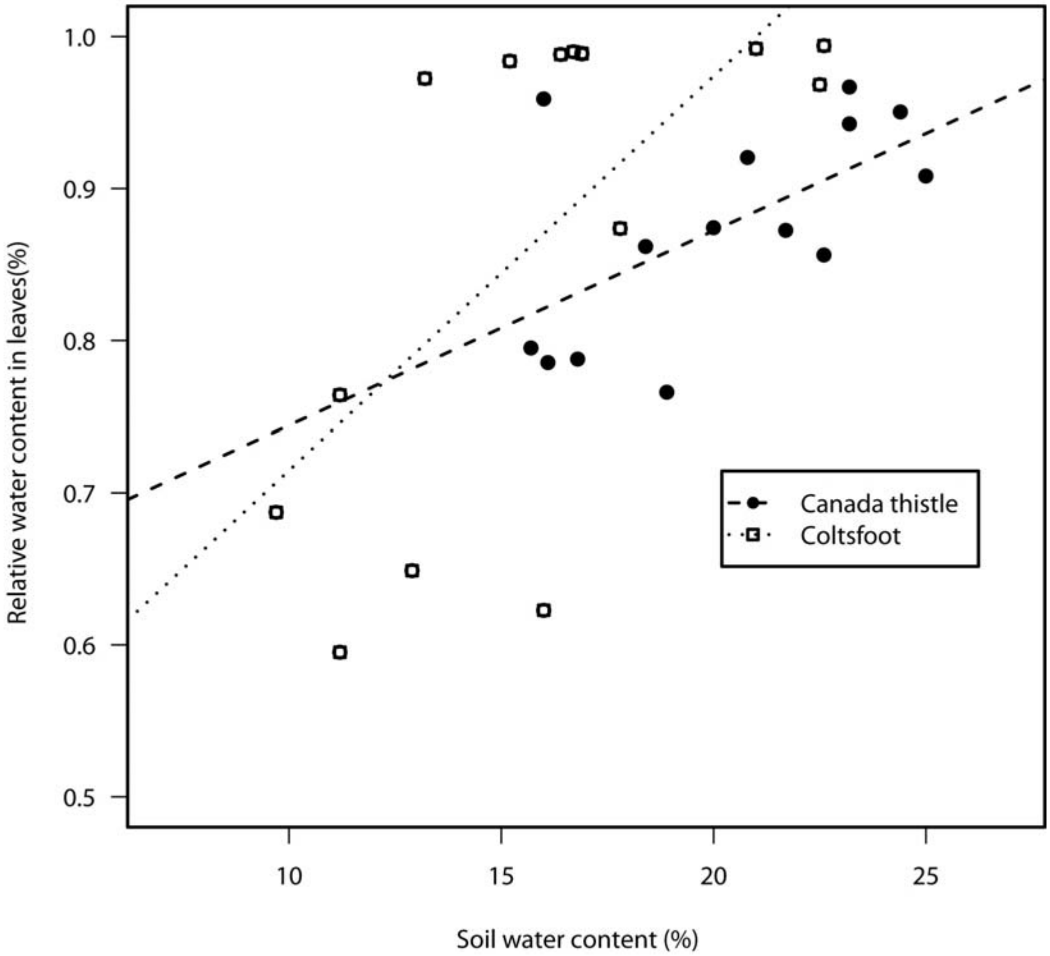

Figure 2. Relationship between relative water content in leaves of broadleaf weeds (RWC) *Canada thistle* and *coltsfoots* and soil water content (SWC) (second experimental run). Regression equations for coltsfoot and Canada thistle were RWC= 0.025 SWC+0.45 and RWC= 0.012 SWC+0.61, respectively; the difference between slopes was barely significant (*P*<0.06).

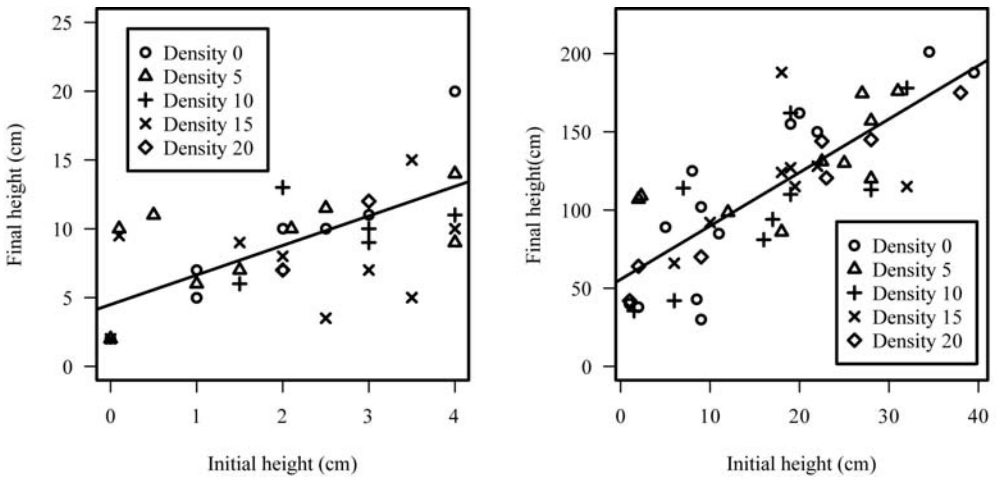

Figure 3. Relationship between the final height (cm) and the initial height (cm) at different cover crop densities of *Canada thistle* (a) and *quackgrass (b)* The regressions were based on sequential test for interaction (see Text). Please note that height of quackgrass t was the sum of shoot length per pot because some rhizomes yield more that one shoot (First experimental run).