Drought effects in dock

Drought Stress alters Solute Allocation in Broadleaf Dock (*Rumex obtusifolius*)

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According to climate models, drier summers must be expected more frequently in Central Europe during the next decades which may influence plant performance and competition in grassland. The overall source-sink relations in plants, especially allocation of solutes to above- and below-ground parts, may be affected by drought. To investigate solute export from a given leaf of broadleaf dock, a solution containing $^{57}$Co and $^{65}$Zn was introduced through a leaf flap. The export from this leaf was detected by analysing radionuclide contents in various plant parts. Less label was allocated to new leaves and more to roots under drought. The observed alterations of source-sink relations in broadleaf dock were reversible during a subsequent short period of re-watering. These findings suggest an increased resource allocation to roots under drought improving the functionality of the plants.

Nomenclature: Broadleaf dock, *Rumex obtusifolius* L. RUMOB.

Key words: Grassland, climate change, water limitation, recovery, phloem transport.

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The current increase in atmospheric CO₂ concentration leads to a number of changes in climate (Meehl et al. 2007). One of the changes projected by climate models is a decrease in summer precipitation and, in general, an increasing frequency of summer droughts in Central Europe (Christensen et al. 2007). Reduced water availability can decrease plant biomass production considerably (Ciais et al. 2005; Peñuelas et al. 2007). As a consequence, the agricultural sector will be affected by increasing drought frequencies in the future (Brown et al. 2011; Fuhrer et al. 2006). Along with yield reductions, changes in climate often lead to changes in the competition between species, e.g. between crops and weeds (McDonald et al. 2009; Patterson 1995a, 1995b).

One of the most troublesome weeds in Europe (Doyle et al. 1984; Gebhardt et al. 2006; Zaller 2004b), broadleaf dock (*Rumex obtusifolius* L.), was found to be less sensitive to drought than the other species (i.e. fodder plants) in intensively managed temperate grassland (Gilgen et al. 2010). Although this phenomenon might be limited to the more humid regions of Europe (i.e. western Central Europe), any increase in competitive ability of broadleaf dock due to climate change will be problematic for farmers. Broadleaf dock is a strong competitor for light and space (fast growth of big leaves) as well as nutrients and water. Roots may grow as deep as 2.5 m (Kutschera et al. 1992). This weed reduces both the quantity (Iijima and Kurokawa 1999; Oswald and Haggar 1983) and the quality (Nashiki et al. 1991) of yield. Since the control of broadleaf dock is very difficult and laborious (see Strnad et al. (2010) for a summary of available methods) an increase in the abundance would cause additional costs for weed management.

Better understanding of the physiological mechanisms behind the observed increase in competitive ability of broadleaf dock against surrounding grassland species under drought would be a prerequisite to an adaptation of management or mitigation of drought effects. It was suggested that broadleaf dock benefits from its deep roots under drought (Gilgen et al. 2010).
In competition with grassland species, broadleaf dock was found to invest into root biomass, thereby building the base for the species’ success in temperate grassland (Zaller 2004a). An efficient supply of carbohydrates to roots of broadleaf dock especially before flowering has repeatedly been detected (Imhoff and Voigtländer 1979; Lang et al. 1975; Voigtländer et al. 1976). However, potential effects of drought stress on these allocation patterns have not yet been studied. In a study of drought and heat effects on temperate grassland forbs it was shown that root growth shapes the community’s response (Dreesen et al. 2012). The allocation of resources to above- and below-ground could thus be a key to understand plant responses to drought. A change in source-sink relations and as a consequence the reallocation of leaf-borne solutes via the phloem to the roots could improve the performance of broadleaf dock under drought.

A suitable technique is needed to investigate the export of solutes from mature leaves to sinks (e.g. roots and growing shoot parts). Radiolabelled heavy metals which are not metabolised may be helpful in this context. Such isotopes were originally used to study the phloem and xylem mobility of heavy metals (Page and Feller 2005; Riesen and Feller 2005; Zeller and Feller 1998). Now that the mobility of the different heavy metals is known, their radionuclides can be used to track phloem and xylem transport of plants. In contrast to organic compounds, heavy metals are not metabolised and not released from plants as gaseous compounds. The radionuclides $^{57}\text{Co}$ and $^{65}\text{Zn}$ can be detected simultaneously in a sensitive manner and are therefore suitable for long-distance translocation studies (Page and Feller 2005; Riesen and Feller 2005). Due to source-sink dynamics we know that a radioactive label fed to a fully expanded leaf can be transported to younger leaves or roots via the phloem (as they both are phloem sinks and need resources like photosynthates or other solutes). From the roots, the solutes (including the radioactive labels) can then be transported to other plant parts with the transpiration stream in the xylem. All label found in older leaves has to be
transported there via xylem as older leaves are phloem sources and not sinks. On the other hand, the redistribution of solutes from fully expanded leaves to growing shoot parts or roots depends on the symplastic transport via the phloem. Good mobility in the phloem was reported for Co and Zn in gramineae (Riesen and Feller 2005) as well as in dicots (Page et al. 2006). Thus, the distribution of radioactive Co and Zn in plants offers an insight into the allocation of resources to the different plant parts. Changes in long-distance transport and in solute allocation caused by drought are reflected in an altered distribution of Co and Zn after labelling a defined leaf with the radionuclides.

To better understand the mechanisms involved in the previously observed rather high biomass of broadleaf dock in grassland under drier conditions (Gilgen et al. 2010), a labelling experiment to track solute transport (i.e. allocation of solutes via the phloem) in this weed was designed. The aim of this study was to understand how the transport of solutes is affected by drought. We hypothesised that the amount of label transported to the roots would increase under drought, as plants would invest more resources into roots to maintain their basic functions.

Materials and Methods

Seeds of broadleaf dock (*Rumex obtusifolius* ssp. *obtusifolius*; originating from the region of Bern, Switzerland) were germinated on coarse quartz sand and grown on deionised water first and later on a standard nutrient solution (according to Page et al. (2012)). At the age of two months, 24 plants were each transferred to a 0.8 L pot with a soil mixture containing 45% Landerde (nutrient rich soil washed off sugar beet grown on the Swiss Plateau), 36% turf, 18% sand and some Seramis clay granules. The pots were randomly assigned to the control or drought treatment before the start of the experiment. Soil water
potential sensors (Watermark soil moisture sensor, Irrometer Company, Inc., Riverside, CA, USA) were placed at the bottom of each drought pot and four of the 12 control pots. The pots were arranged on two shelves in a climate cabinet and positions were randomly rotated every week. The cabinet was set to a 14 h day at 24°C and a 10 h night at 16°C. Light was supplied with 55 W lamps and adjusted to a level of around 100 to 120 µmol m⁻² s⁻¹ at leaf level. Deionised water was supplied regularly to keep the pots well saturated. The evapotranspiration (i.e. the water loss) of every pot was assessed gravimetrically by weighing the pots before and after watering (every second or third day).

The labelling solution containing the radionuclides \(^{57}\)Co and \(^{65}\)Zn was introduced into the leaf via a flap in the petiole. The method described by Schenk and Feller (1990) was adapted to the different leaf morphology of dicots. A test prior to the experiment had shown that cutting the petiole longitudinally in the middle through the symmetry axis and using one of the two equal parts as the flap resulted in the best uptake of liquids. After 46 days of growth on soil, an approximately 4 cm long flap was cut into the petiole of the youngest fully expanded leaf (in general the 10th or 11th leaf). This flap was positioned in a tube containing 0.8 ml of the radionuclides \(^{57}\)Co and \(^{65}\)Zn dissolved in 10 mM RbCl and 10 mM SrCl₂. If necessary, the flap was repositioned in the remaining liquid after 48 h. All except two (one drought and one control plant) of the 24 plants took the solution up completely. After approximately 96 h, the tubes were recovered for later verification of label uptake (see below). All plants were watered before the label was applied to make sure that the uptake of label was not confounded by the treatment. Following that, the 12 drought plants did not receive water any longer while the 12 control plants were still watered as before.

Seven days after the application of the label and the last watering of the drought plants, four randomly chosen plants from both treatments (well watered control vs. drought) were harvested. Of the remaining eight drought plants, four were kept at drought conditions
while the other four were re-watered. The watering of the eight control plants remained unchanged. Four plants from each of these three groups (control, drought, re-watered) were harvested after another seven days. Four control plants were used for evapotranspiration measurements only but were not analysed further.

The labelled petiole and leaf blade were sampled separately while the other leaves were sampled as a whole. All dead leaves were pooled in one sample as were the side shoots. Roots were washed from the soil and also sampled. For practical reasons, the base (lowest part of leaves and uppermost part of roots) was sampled separately. Dry weight (after drying at 60°C for 24 h) of the different samples was measured.

The dried plant samples as well as the recovered labelling tubes and a tube containing 0.8 ml of the labelling solution (i.e. a reference tube) were analysed with an automatic gamma counter (1480 Wizard 3”, Wallac, Turku, Finland) recording gamma radiation emitted by $^{57}$Co and $^{65}$Zn at the same time. Counting duration was set to 60 min and results are expressed as counts per minute (cpm) per sample.

To ensure that no contamination with the label had occurred, Sr content in the different plant parts (as described above) was also assessed. Sr is immobile in the phloem and should therefore only be found in the labelled leaf. Sr content was measured by atomic absorption spectrometry (SpectrAA 220FS, Varian Techtron, Mulgrave, Australia). Once gamma counting was finished, samples were ashed at 550°C for several hours. After cooling 0.2 ml 10 N HCl and subsequently 2 ml deionised water were added to the ash. An adequate dilution with 5000 ppm LaCl$_3$ in 0.1 N HCl was used to assure all samples fit the measurement range of the instrument (0-8 ppm). To quantify the background content of Sr originating from the soil, an additional set of six control plants was grown under the same conditions and analysed for Sr content as well.
The effect of the treatment on evapotranspiration and soil water content was tested using one-way ANOVA. For statistical analysis, leaf samples were pooled in groups: labelled plant parts (labelled petiole and leaf blade), leaves that were older than the labelled leaf (including dead leaves; in the following called “old leaves”), leaves that were younger than the labelled leaf but present and not fully expanded at the time of labelling (“young leaves”), leaves that emerged after labelling (“new leaves”), leaves from additional shoots (“side shoots”) and below-ground parts (roots and base, in the following referred to as “roots”). In general, four independent replicates in a fully randomised design were analysed for each treatment and each harvest date. However, one of the drought replicates was excluded from all analyses on harvested plants as the plant only took up approximately 75% of the labelling solution. The effect of the treatment was tested for the two harvests separately using ANOVA. The different treatments within the respective harvest were compared using a LSD test. All statistical analyses were performed with R 2.14.2 (R Development Core Team 2012).

**Results and Discussion**

Withholding water significantly reduced evapotranspiration within four days (Fig. 1). While control plants lost 65.03 g H$_2$O d$^{-1}$ (±3.65 g H$_2$O d$^{-1}$; mean±SE, n=12), drought stressed plants lost seven times less water (9.31±0.72 g H$_2$O d$^{-1}$) at the end of phase 1. Evapotranspiration significantly increased in response to re-watering. However, evapotranspiration of re-watered pots did not reach the level of control pots after one week. Evapotranspiration of drought pots further decreased during phase 2 and reached almost zero at the end of the experiment. Soil water potential progressively declined during phase 1. In contrast to evapotranspiration, soil water potential immediately recovered once pots were re-watered (Fig. 1).
Total dry weight was not significantly affected by one week of drought (3.45±0.23 g in drought plants compared to 3.73±0.44 g in control plants, mean±SE, n=4; p=0.60). Only the dry weight of new leaves was significantly reduced by drought at the first harvest. This was also reflected in the relative contributions of the different plant parts to the total plant biomass (Fig. 2).

At the second harvest, total dry weight of drought stressed and re-watered plants was similar (3.55±0.45 g and 3.35±0.13 g, respectively; mean±SE, n=3-4) but significantly lower than total dry weight of control plants (4.84±0.24 g; mean±SE, n=4). Control plants had been able to increase their biomass (i.e. their total dry weight) during phase 2, while total dry weight of drought stressed and re-watered plants remained constant. The lower total dry weight of drought stressed and re-watered plants was mainly caused by a decrease in root dry weight and a significantly decreased dry weight of new leaves in drought plants and old leaves in re-watered plants, respectively (Fig. 2). Thus, while one week of re-watering was not enough for root biomass to recover, it allowed growth of new leaves and the accumulation of similar dry weights in new leaves as in control plants. The production of new leaves indicates that one week of drought stress did not irreversibly damage the plants. This enormous potential of broadleaf dock to withstand unfavourable conditions and recover quickly is clearly underlined by the relative contributions of the different plant parts to total dry weight (Fig. 2). There was no difference between control and re-watered plants in the relative biomass invested in any of the plant parts. In contrast, drought stressed plants attributed significantly less biomass to new leaves while significantly more biomass was concentrated in old leaves (that were mostly dead) compared to plants from the other two groups. However, it is remarkable that the proportion of root dry weight was unaffected by the treatment (Fig. 2).
The labelling solution (with 855 cpm $^{57}$Co and 257 cpm $^{65}$Zn fed to each plant) was almost completely taken up (largest rest remaining in the tube <10% for both $^{57}$Co and $^{65}$Zn). The phloem-immobile Sr was detected in the labelled leaf, but was not above the background in the other plant parts, indicating that no contaminations had occurred (data not shown). An average label of 840±9 cpm $^{57}$Co and 265±4 cpm $^{65}$Zn (mean±SE, n=19) was recorded in the plants. A significant treatment effect was only observed in phase 2 when significantly lower activities of $^{57}$Co and $^{65}$Zn were detected in drought stressed plants. Most of the label was retained in the labelled leaf and only between 5.5% and 9.4% of the $^{57}$Co and between 5.5% and 14.1% of the more mobile $^{65}$Zn were exported to other plant parts. However, the activity transported (on average 54±6 cpm and 21±3 cpm for $^{57}$Co and $^{65}$Zn, respectively; mean±SE, n=19) was still high enough to analyse the allocation to roots and other shoot parts. There were no significant treatment effects on absolute or relative amounts of activity transported, except for $^{65}$Zn in the second harvest. Significantly more $^{65}$Zn was transported out of the labelled leaf of re-watered plants compared to drought stressed plants. A similar, but not significant, trend was observed for $^{57}$Co (data not shown).

A high fraction of the transported label was detected in new leaves and roots, where also the main treatment effects were observed (Fig. 3). The fraction of label transported to new leaves tended to decrease in response to drought, while higher levels of $^{57}$Co and $^{65}$Zn were detected in the roots of these plants. In re-watered plants, the fraction of label in new leaves and roots became again very similar to control plants indicating a reorganisation of the source-sink relations during the recovery phase. The fraction of label transported to the roots was higher in drought stressed compared to control and re-watered plants even though the root biomass of drought stressed plants was lower. No difference between treatments at any of the two harvests was found in the other three plant parts (young leaves, old leaves and side shoots; data not shown). Zaller (2004a) suggested that the success of docks under competitive
conditions is caused by their ability to invest into roots. Our observation that in broadleaf dock more phloem-mobile solutes were directed to the roots under drought are consistent with this concept. The additional resources directed to roots were obviously not used to grow new root biomass (root dry matter decreased under drought) but for maintenance (relative contribution of root dry matter to total biomass was constant) and as storage for later regrowth (resources allocated from roots to new leaves under re-watering).

From the experiment reported here it became evident that source-sink relations, and as a consequence solute allocations, in broadleaf dock are strongly affected by a drought period. However, the changes can be reversed quite rapidly during a subsequent recovery phase. Modifications in the redistribution pattern during a drought period as well as during a subsequent recovery phase may improve the overall performance of a species. It must be borne in mind that the persistence of broadleaf dock is controlled by many different factors like water but also nutrient availability (Hann et al. 2012; Hejcman et al. 2012; Humphreys et al. 1999; Křišťálová et al. 2011) or management (Hopkins and Johnson 2002; Martinkova et al. 2009). Possible interactions of these factors remain to be investigated in the future. In a community, the persistence of broadleaf dock will also depend on the presence and identity of neighbours and their behaviour in competition. To date, no other species were studied using the method presented here. Results from experiments solely focusing on above- and below-ground biomass responses to drought are contradictory, showing either a decrease (e.g. Weisshuhn et al. 2011) or an increase (e.g. Dreesen et al. 2012) in the share of biomass invested into roots under drought. Nevertheless, the ability to recover quickly after drought might improve the performance of broadleaf dock under future drier conditions, at least in the more humid regions of Central Europe.

From a more general point of view, it can be concluded from the findings reported here that analysing the transport of radioisotopes of phloem-mobile heavy metals (e.g. $^{57}$Co
and $^{65}$Zn) after their introduction into a defined leaf is a sensitive and suitable technique to detect changes in the source-sink network. Questions related to impacts of abiotic stresses on the whole plant level (e.g. in the context of climate change) can be addressed with this method.

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Figures Legends

Figure 1. Average daily evapotranspiration and soil water potential of pots with a broadleaf dock plant. In phase 1 water was withheld from pots of the drought and re-watered treatments. In phase 2 only drought plants were not irrigated. Averages and standard errors are shown (n=4-12).

Figure 2. Absolute and relative dry weights of plant parts (excluding the labelled leaf) at the two harvests (i.e. at the end of phase 1 and phase 2). Old leaves were older than the labelled leaf, young leaves were younger than the labelled leaf but already present at the time of labelling, new leaves were formed after labelling and side leaves are leaves from additional shoots (all age classes). Averages and standard errors are presented (n=3-4). Within each harvest significant treatment differences are shown by different letters.

Figure 3. Relative amounts of $^{57}$Co and $^{65}$Zn transported into new leaves and roots expressed in % of the transported label. New leaves were formed after labelling. Averages and standard errors are presented (n=3-4). Within each harvest significant treatment differences are shown by different letters.
Figure 1

[Graph showing daily evapotranspiration and soil water potential over days after start of treatment, with control, drought, and re-watered conditions indicated.]

Days after start of treatment

Days after start of treatment

Phase 1

Phase 2
Figure 2
Figure 3