Growth and sprouting dynamics of the invasive alien species warty cabbage (*Bunias orientalis*) during the growth season

Vekst og regenerering hos den fremmede invaderende arten russekål (*Bunias orientalis*) gjennom vekstsesongen

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Abstract

Alien species are considered as one of the greatest drivers of biodiversity loss. In Norway Warty cabbage (*Bunias orientalis* L.) is considered to be a species that has a negative impact on indigenous biological diversity.

In order to eradicate an unwanted plant species as efficiently as possible it is important to take action at the stage where it is at its weakest and most vulnerable. Perennial vascular plants are weakest and prone to damage at the point in their seasonal life cycle where they have a minimum of energy stored in their below ground plant parts, called the compensation point.

In this thesis I have been studying plants of *B. orientalis* in order to locate a possible dry-weight minimum in the below ground plant parts, the transport direction of carbohydrate reserves by using ^{14}C, the taproot's ability to produce aerial shoots from adventitious buds and the time of seed ripening. The experiments were carried out from September 2009 to March 2011, and the studies were done on both young (1-2 years old) transplanted plants at Ås and on older plants sampled directly from a *B. orientalis* population along the E6 in Oslo.

In young plants (1-2 years old) of *B. orientalis* no dry-weight minimum of the below ground plant parts was found at any specific development stage, but from the isotope experiment changes in the transport direction of carbohydrate reserves were detected. At the stage where the smallest proportion of ^{14}C was located in the roots, might be the stage where the source-sink dynamic of carbohydrate reserves shifts from the below ground plant parts being the source to being the sink, and a weak point in the species seasonal life cycle. At this point plants in their generative stage had started stem elongation and had an average height of 31 cm and inflorescence with the first individual flowers visible, but still closed. The vegetative plants had medium sized rosette with an average rosette height of 28 cm and an average diameter of 39 cm.

The young plants at Ås did not show a defined drop in their regenerative capacity from root sections at any point, but for the older plants a minimum of regenerative capacity from root sections was found at the stage where the plants had started to elongate and had an average height of 26 cm and visible inflorescence. This development stage corresponds well with the development stage found in the isotope experiment where an indication of a weak point was found.

In order to find the time of seed ripening, seeds were harvested from the roadside population in Oslo on the 6th and 20th July and on the 3rd and 17th of August. The seeds harvested in the beginning of July did not manage to germinate, while the seeds had reached maturity in the second half of July.

B. orientalis is an extremely vigorous plant with a powerful taproot which make weed control difficult, and it is therefore of great importance to monitor this species and to take action against new occurrences as soon as they are detected.

Sammendrag

Fremmede invaderende arter er ansett som en av de største truslene mot biologisk mangfold. Norsk Svarteliste 2007 gir en oversikt over økologiske risikoanalyser for et utvalg fremmede arter i Norge. Russekål (*Bunias orientalis* L.) er vurdert å utgjøre høy risiko for stedegent biologisk mangfold.

I bekjempingen av uønskede arter er det viktig for å oppnå best mulig resultat å kunne utføre tiltak på det tidspunktet der arten er svakest mot kontrolltiltak som luking, nedkapping eller sprøyting. Flerårige karplanter er svake og mer mottaglige for ytre påkjenninger på det utviklingsstadiet hvor de har et minimum av opplagsnæring i de underjordiske plantedelene, kalt kompensasjonspunktet.

I denne oppgaven har jeg studert russekål for å finne et eventuelt tørrvektminimum i de underjordiske plantedelene, transportretning for opplagsnæring, rotas regenereringsevne etter oppdeling og tidspunkt for frømodning. Forsøkene ble gjennomført i tidsrommet september 2009 til mars 2011 på unge (1-2 år) omplantede planter på Ås og eldre planter høstet direkte fra en populasjon langs E6 ved Mortensrud i Oslo.

Det ble ikke funnet noe tørrvektminimum eller tendenser til fall i tørrvekten i de underjordiske plantedelene ved noe utviklingsstadium hos de unge omplantede plantene på Ås. Men etter å ha tilført ^{14}C til planter på forskjellige utviklingsstadier og funnet det igjen, kunne jeg identifisere transportretningen av CO_2 i plantene etter hvor i planten ^{14}C ble funnet. Basert på disse funnene har russekål trolig et minimum av opplagsnæring på det stadiet der generative planter har startet strekningsveksten og vegetative plantene har medium stor rosett. Plantene i den generative fasen hadde på dette tidspunktet en gjennomsnittshøyde på 31 cm og

blomsterknopper med de første enkeltblomstene synlig. De vegetative plantene var gjennomsnittlig 28 cm høye og hadde en gjennomsnittlig diameter på 39 cm.

Hos de eldre russekålplantene fra populasjonen i Oslo fant jeg et fall i deres vegetative regenereringsevne fra 5 cm lange rotbiter når plantene hadde en gjennomsnittlig høyde på 26 cm og synlig blomsteranlegg. Dette utviklingsstadiet samsvarte godt med det utviklingsstadiet som indikerer et minimum av opplagsnæring funnet ved bruk av ^{14}C. Selv om de unge, omplantede russekålplantene på Ås ikke viste noen nedgang i evnen til å regenerere vegetativt fra rotbiter gjennom vekstsesongen, er det likevel sannsynlig at det for eldre planter er en sammenheng mellom artens kompensasjonspunkt og rotbiters skuddskytingsevne.

For å finne tidspunkt for frømodning ble frø samlet inn 6. og 20. juli samt 3. og 17. august 2010. Frø høstet 6. juli ble ikke funnet å være spiringsmodne, mens frø høstet 20. juli hadde høyest spiringsprosent og var minst spiretrege.

Russekål er enormt livskraftig og den kraftige påleroten gjør den vanskelig å bekjempe, det er derfor viktig å overvåke denne arten og iverksette tiltak så snart nye forekomster oppdages.

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1. Introduction

"Invasive alien species have affected native biodiversity in almost every type of ecosystem on Earth. As one of the greatest drivers of biodiversity loss, they pose a threat to ecosystem integrity and function and therefore, to human well-being" (Convention on Biological Diversity 2009).

Under the Convention on Biological Diversity, Norway is under obligation to as far as it is possible and practical to prevent introduction, control or eradicate alien species which are threatening ecosystems, habitats or other species (MD 2007).

As a result of St.meld. nr 21 (2004-2005) about the Government's environmental politic and the environmental status in the country, "Tverrsektoriell nasjonal strategi og tiltak mot fremmede skadelige arter" were made and signed by ten ministers, and the 2007 Norwegian Black List (Gederaas et al. 2007) were made. Naturmangfoldloven (2009) imposes upon everyone a principle of general care (§6) and a precautionary principle (§9) to protect the biodiversity. This means that everybody has a responsibility regarding the spread of alien species which can impose a threat on biodiversity.

2007 Norwegian Black List is the first official overview of ecological risk analyses for a selection of alien species in Norway. Risk analyses were carried out on 217 of the 2438 alien species listed. The analysed species were divided into three categories: low risk, unknown risk and high risk. 17 species of vascular plants were placed in the high risk category and *Bunias orientalis* L. (*Brassicáceae*), the subject plant of this master thesis, is one of them.

1.1. *Bunias orientalis*

The native range of *B. orientalis* is Caucasus and southern Russia (Birnbaum 2006). The species has been introduced and is naturalized in many countries in Europe, including Norway (Birnbaum 2006) where it was first recorded in 1812 (Korsmo 1954). The first introduction was most likely related to import of Russian grain (Korsmo 1954).

B. orientalis has spread within the past 200 years and is still spreading extensively throughout Europe. Until the late 1900s only a few occurrences of the species were found, but for the last 20 years the number of sites have increased enormously (Birnbaum 2006). In Norway the plant is now commonly naturalised and is still spreading in the south-east part of the country and

along the coast to at least 64° N (Nord-Trøndelag) (Lid et al. 2005). Korsmo (1954) and Korsmo et al. (1981) describe *B. orientalis* to be widely distributed on grassland, waste ground, places of ballast, along roadsides and railways, and occurs as a weed in meadows, pastures, yards etc, but the experience today in Norway is that this species is rarely found on farmers grassland or pastures (Båtvik 2011).

B. orientalis is a semi-rosette, hemicryptophyte (Def:. a plant with perennating buds situated at or just below the soil surface), polycarpic, perennial herb that can attain ages of more than 12 years (Dietz et al. 1999a). The species flower in the second year or later (Dietz & Ullmann 1998). In the first year the plant develops a taproot of up to 30 cm long and a small rosette, in the second year the plant develops a single flower stem and the taproot continues to grow and develops adventitious buds. In the third year the root develops a several headed shape, and in the fourth year, the plant develops numerous branched vigorous flower stems (Korsmo 1954). Rosette leaves of an adult plant can be up to 60 cm long and 10 cm wide (Korsmo 1954), the rosette diameter 10-100 cm (Dietz et al. 1999a) and the shoot can reach a height of up to 2 meters (Steinlein et al. 1996). At nutrient rich sites the plants will develop lager rosettes than at nutrient poor sites (Steinlein et al. 1996).

B. orientalis flower in June – July followed by seed ripening in July – August. The flowers are bisexual. Fruits normally contain 2 seeds whit a nut like hardness (Korsmo 1954). According to Steinlein et al. (1996) the fruits may be viable for more than 3 years. The number of fruits per plant varies from 200-5000 (Korsmo 1954). In order for the seeds to germinate, the pericarp of the fruit must soften for the moisture to reach in to the seeds. This normally takes some time and the result is slow germination. Without disturbance the plants can only reproduce by seeds (Korsmo 1954), and are able to build a dense seed bank of up to 390 fruits per litre of soil (Steinlein et al. 1996). According to Dietz et al. (1999a) seed can germinate without a dormancy period following dispersal in autumn.

Korsmo (1954) found a lower germination rate among seeds wintered indoor under dry conditions than among seeds wintered outside in the soil. He also found that seeds sown at a depth of 0.5 – 3 cm had higher germination rate than seeds sown deeper or at the soil surface. The highest germination rate was attained at 1 cm depth. Shallow sowing, 0 cm, and deep sowing, 7 and 8 cm, gave no germination (0%). However Dietz et al. (1999b) found that seeds

from fruits planted at a depth of 20 cm managed to germinate. Steinlein et al. (1996) found that the germination rate increased with higher soil humidity, nitrogen availability and increased mowing intensity in a *B. orientalis* population.

In a Swedish experiment where they were examining seeds response to short-duration light exposure, seeds of *B. orientalis* were cold and wet stratified in the dark for 18 weeks before being subject to 12 hrs light per day, 24 hrs darkness or only 5 s light exposure. The results showed that seeds of *B. orientalis* had no response to short-duration light exposure and germinated regardless of the light conditions (Milberg et al. 1996).

The taproot of a fully developed *B. orientalis* plant have been found to be up to 1.6 meter long and with a diameter of 6 cm at the top (Korsmo 1954).The taproot produces vegetative offspring from dormant buds if the root is damaged or severed anywhere beneath the root head, and if the root is parted into smaller pieces, new plants can develop from adventitious buds on the root fragments (Korsmo 1954). According to Steinlein et al. (1996) can root fragments from *B. orientalis* retain a regeneration potential of 50 % after a 30 % water loss. Even after a separating of the root cortex from the root stele, retained the cortex a regeneration potential of 50 % and the stele a regeneration potential of 30 % (Steinlein et al. 1996). Root fragments planted at a depth of 20 cm from the soil surface retained their ability to produce aerial shoots (Dietz et al. 1999b; Steinlein et al. 1996), even fragments as short as 2 cm were found to regenerate at those depths, while 1 cm long fragments did not manage to regenerate at all (Dietz et al. 1999b).

Young plants of *B. orientalis* are poor competitors when grown in mixture with taller forbs (Dietz et al. 1998), but the species seems to suffer less from competition in grass dominated vegetation (Dietz et al. 1999a). Results from field experiments in Germany shows that a moderate mowing regime of 2-3 times a year had no effect on reducing populations of *B. orientalis*. Rosette growth was in fact promoted by higher mowing intensity in all study sites (Steinlein et al. 1996). Dietz and Ullmann (1998) found that the dominance of young plants and the proportion of flowering young plants were positively related to frequency and intensity of mowing. Frequently mowed habitats were dominated by 1 and 2 year old plants, as opposed to rarely disturbed habitats (un-mowed) which had a higher proportion of older (≥4 years old) individuals. Most plants in frequently mowed habitats were reproductive, including 1 and 2

year old plants, and plants in the reproductive stage were younger in frequently disturbed habitats than in rarely disturbed habitats (Dietz & Ullmann 1998).

By studying the invasion history of a 9 year old roadside population of *B. orientalis* Dietz et al. (1999a) found that the features of the population fits the pattern expected of a viable, expanding population of an invasive plant species. The population had rapid growth which resulted in a broad age structure and a continuously increasing occupation of the available area. Moderate disturbance of irregular mowing had most likely promoted population increase, advancing both seedling establishment and growth of adult plants. Some one year old plants, most two year old plants and all older plants were reproductive (Dietz & Ullmann 1998). No dead roots of *B. orientalis* were found during the studies of these sites, which indicate low mortality of established plants (Dietz et al. 1999a).

1.2. Weed classification and biology

Korsmo (1954) classified weed species into different biological groups based on their life-span, mode of reproduction and characteristics of the root system. Based on the plants life-span, he divided them into four main biological groups; annuals, winter annuals, biennials and perennials. The perennials are again divided into two groups, creeping perennials and stationary perennials. The creeping perennials can extend horizontally by means of creeping vegetative organs, while the stationary perennials cannot extend or can only slightly extend from the spot where they originally established. Plant species in the biological group stationary perennials are not able to regenerate on their own other than with seeds, but some species in this group have the ability of vegetative regeneration from fragments from the root and sometimes also from the stem. The stationary perennials are further divided into four sub-groups based on their root system; fibrous root, rootstock, taproot and false root. *B. orientalis* is classified as a stationary perennial with taproot. Other weed species in the same biological group are e.g. docks (*Rumex* spp.) and *Taraxacum* spp.

In weed control it is of importance to know and understand the species biology as well as plant ecology in order to control the species in the most efficient way. The biological classification system described above is one remedy, but in addition, knowledge about the growth pattern during the growth season for both disturbed and undisturbed plants is of vital importance. Examples of important aspects include depth of root systems, distribution of dormant buds

including where they are and what triggers them to sprout, as well as external and internal dormancy in both seeds and roots.

The compensation point for a perennial plant can be defined as the time where the source-sink dynamic of carbohydrates reserves shifts from the underground plant parts as the source and the above ground plant parts as the sink, to the reverse (Teasdale et al. 2007). This occurs before the total leaf areas are large enough to enable photosynthesis to compensate for the consumption of substances by respiration (Håkansson 2003).

Compensation point has been found in several weed species, but mainly in creeping perennials like *Elytrigia repens* (Håkansson 1974), *Sonchus arvensis* (Håkansson 1969) and *Circium arvense* (Gustavsson 1997). But some studies, at least related to this theme, have been done on stationary perennials like *Rumex* spp. (Fykse 1986) and *Artemisia vulgaris* (Oliver 2008). Fykse (1986) studied the sprouting ability from root sections of three different dock species (*Rumex* spp.) as well as the dry weight, and found that the root sections ability to produce aerial shoots increased markedly with increasing weight. The result from this study and other studies indicates that there is a correlation between the compensation point and the regenerative capacity, studying the regenerative capacity may therefore be used as a method of finding the compensation point. Such correlation is also found in other weed species, e.g. *S. arvensis* (Fykse 1974). Traditionally dry-weight minimum of the below ground plant parts, correlated with assessments on the shoot stage, is used to identify the compensation point. Another method is to follow the CO_2 transport in the plant.

1.3. Technique for determination of ^{14}C

Liquid scintillation counting is a standard laboratory measurement technique applied within the life-sciences for measuring radiation from beta-emitting nuclides such as carbon-14 (^{14}C), phosphorous-32 (^{32}P) and tritium (^{3}H).

Liquid scintillation counters are an efficient and practical means of quantifying beta radiation (β-radiation). The liquid scintillation counter measures the ionizing radiation of the sample. The sensor of the instrument consists of a transparent crystal, usually phosphor, plastic or organic liquid that fluoresces when struck by ionizing radiation. A sensitive photomultiplier tube, which is coupled to an electronic amplifier will count and quantify the amplitude of the signals produced by the photomultiplier, thus measuring the light from the crystal.

A liquid scintillation solvent, commonly named scintillation cocktail, is a mixture of an aromatic solvent and small amounts of other additives known as scintillators (fluors). β-particles emitting from the sample transfer energy to the cocktail molecules (fluors) which are excited. The excited molecules then dissipate the energy by emitting light. Each β-emission results in a pulse of light, measured by the liquid scintillation counter.

1.4. Objective

The main purpose of my thesis was to increase the knowledge about the biology of *B. orientalis.* My hypotheses were;

(1) The taproot of *B. orientalis* has a weak point at a certain development stage in the plant's seasonal life cycle.
(2) The regenerative, vegetative capacity of buds from the taproot varies throughout the season and is closely related to the dry-weight minimum of the root, the compensation point.
(3) Seeds from cut plants in the early phase of seed ripening will not germinate.

My goal has been to make this knowledge as useful as possible for anyone involved in weed control of areas infested by *B. orientalis.*

2. Materials and methods

2.1. Experimental locations and plant material

The field experiments took place at Ås (59°40' N, 10°46' E, 90 m asl) from 5 May to 16 June 2010 and at Mortensrud, Oslo (59°50' N, 10°50' E, 150 m asl) from 6 May to 17 August 2010. The greenhouse experiments took place at Ås from February 2010 to Mach 2011. The daily precipitation and middle temperatures for Ås in the period 8 September 2009 to 16 June 2010 and in Oslo in the period 6 May to 17 August 2010 are presented in figure 1 and 2, respectively.

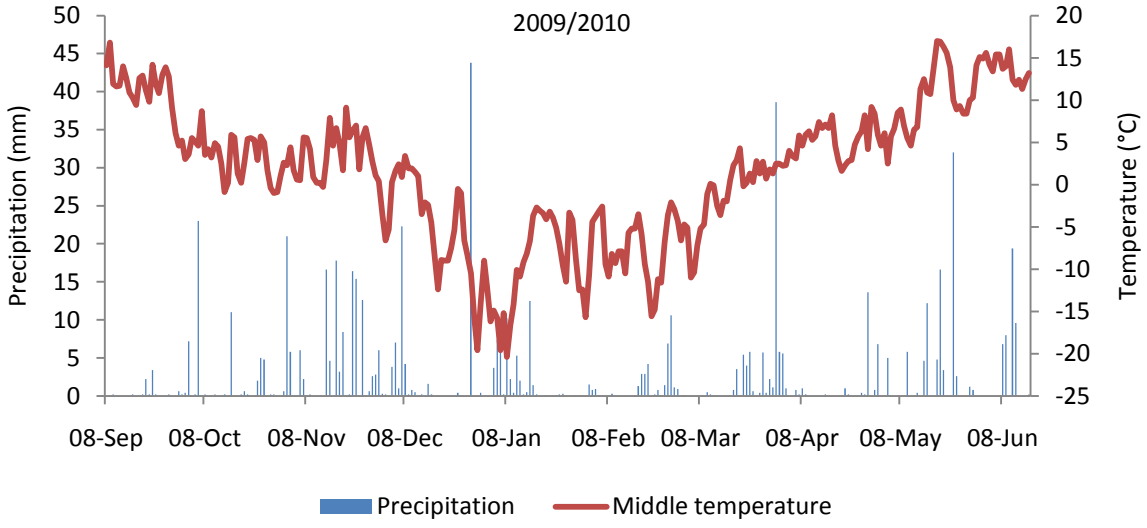

Figure 1 Daily precipitation (mm) and middle temperatures (°C) at Ås from time of transplanting to harvest

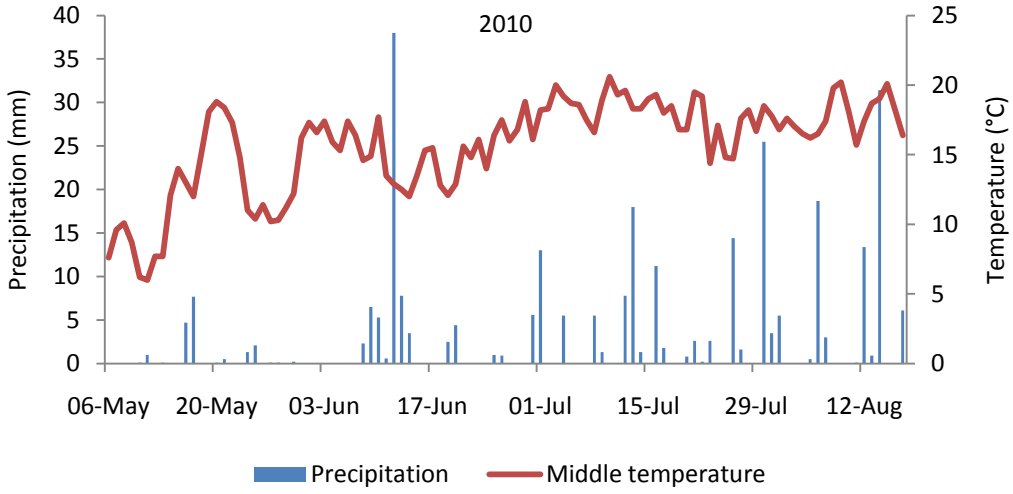

Figure 2 Daily precipitation (mm) and temperatures (°C) in Oslo during the experimental period

Plant material for all experiments was sampled at Mortensrud, Oslo. 300 juvenile plants of *B. orientalis* were sampled 4 and 7 September 2009 from the roadside population at Mortensrud. The size and the small rosette of the sampled plants indicated that they had germinated from seeds, or sprouted from root fragments during spring 2009. Some of the larger roots originated most probably from root fragments of older plants.

Prior to transplanting, the sampled plants were stored outside for 3 days under a cover of grass and soil, and moist paper was wrapped around the roots to prevent them from drying.

2.2. Field experiment at Ås, transplanted plants

The plants were grouped visually, according to the size of the roots, into six replicates: Rep. 1 and 2; small, rep. 3 and 4; medium towards small, rep 5; medium towards large and rep 6; large.

264 plants were planted in a loam-sandy soil at Kirkejordet, Ås on 8 and 9 September 2010. The plants were planted in replicates in 11 rows with 24 plants in each. The row distance was 75 cm and the distance between the plants was 50 cm. Field map is presented in appendix 2. The plants were not watered during the experiment, but left to the natural rainfall. Other weeds were controlled by hand weeding.

182 roots (approx. 70 %) sprouted by May 2010. Because of the low survival rate and a relatively poor standard of the specimens, plant harvesting was restricted to four times: 1) 5 May, 2) 19 May, 3) 4 June and 4) 16 June, 2010.

The shoot and root of two randomly chosen plants from each replicate, in total twelve plants, were harvested each time. Prior to harvesting, measurements of plant height (without stretching the leaves, and all plants 5 cm or lower, were registered as ≤ 5 cm), plant diameter, number of unfolded leaves and the length of the longest leaf as well as plus/minus flowering were recorded . BBCH classification system (Hess et al. 1997) were used to describe the inflorescence and flowering. A complete overview of the BBCH codes used in this thesis is given in appendix 1. After harvesting, the roots and aerial plant parts were separated and fresh weight recorded. All lateral roots were removed from the main taproot, before the taproot was cut in to sections of 5 cm. Due to short roots, root section shorter than 5 cm was included. The fresh weight of all root sections was recorded. For the dry weight (DW) measurements, one

root from each replication (6 roots) was dried for 72 hrs at 60 °C. The diameter of the remaining root sections was recorded, and roots were planted individually in organic soil (35 % organic matter) at a depth of 1 cm. Details of soil nutrient concentration and pH and are presented in table 1. The pots were placed in the greenhouse for 21 days for the testing of sprouting ability.

Table 1 Soil nutrients

L.O.G Gartnerjord			
pH	phosphorus	potassium	nitrogen
5.5 – 6.5	35 mg/l	170 mg/l	850 mg/l

2.3. Field experiment in Oslo, roadside population

Six plants of *B. orientalis* were harvested every second week from the roadside population at Mortensrud, Oslo. Plants were harvested 8 times in the period from 6 May 2010 to 17 August, 2010. Only plants in the generative stage were harvested. After the fourth harvest (22 June), part of the population were cut at a height of 15-20 cm with a grass trimmer, and the following four harvestings were only from the cut plants. The plants were harvested by digging them up manually with a spade. The aim was to harvest plants with the complete root, but because of extremely long taproots and stony soil, it was impossible to extract the whole length of the root without loss.

The plants development stage, including height, rosette diameter, inflorescence/flowering according to the BBCH classification system and number of flower stems were recorded at each harvest. The shoots were separated from the roots, dried for 72 hrs at 60 °C and weighed. All lateral roots were removed before the main taproot was cut into sections of 5 cm. Root sections shorter than 5 cm were not included. An identical procedure to that of field experiment Ås was followed for testing sprouting ability.

2.4. Greenhouse experiment

The root sections sampled at Ås and Oslo were grown for 21 days. The growth conditions were 16 hrs day (200 µmol m^{-2} s^{-1}) and 8 hrs night, temperature was 18 °C day and 12 °C night at a constant 70 % humidity. The plants were watered when needed. At harvest the number of above and below surface aerial shoots from each root section were counted. New leaves with an individual attachment to the root section counted as one shoot. Shoots from thin side roots

were not counted, because it was impossible to know if the side root had developed during the experimental period.

2.5. Isotope experiment

Twenty five plants of equal size similar to replicate 3 and 4 were planted in 5 litre containers containing L.O.G. Gartnerjord (8 September 2009). After planting, the plants were placed outdoor for a few weeks before they were placed in the dark in a cooling chamber at 2-4 °C. The plants were transferred to a greenhouse in January 2010 with the same growth conditions as described for the greenhouse experiments. The plants were watered with tap water when needed.

The first four plants were exposed to $^{14}CO_2$ in February 2010. Each $^{14}CO_2$ experiment lasted for 7 days. Height (leaves were stretched before measuring), rosette diameter, number of unfolded leaves, the length of the longest leaf and inflorescence according to BBCH were recorded on all plants at the beginning and after the exposure period. The $^{14}CO_2$ exposures experiments were repeated 6 times (Table 2).

Table 2 Dates for exposure and development stages

Exp.	Exposure date	Harvest date	Number of cold treatments	Exposed plants (plant No)	Development stage V=vegetative G=generative
1	12.02.10	19.02.10	1	4 (1, 2, 3, 4)	1, 2 and 3: Small rosette (V) 4: Elongation/inflorescence (G)
2	03.03.10	10.03.10	1	4 (6, 7, 19, 9, control plant)	6, 7 and control: Medium rosette (V) 9 and 19: Flower (G)
3	25.03.10	01.04.10	1	2 (8, 11)	Large rosette (V)
	Cold treatment period				
4	09.07.10	16.07.10	2	2 (18, 20)	18 and 20: Rosette 20: Inflorescence
5	16.07.10	23.07.10	2	2 (12, 13)	Flower
6	22.07.10	29.07.10	2	2 (15, K2)	Elongation/inflorescence

After the third exposure period, none of the remaining plants had generative growth. Therefore, all remaining plants were cut down and stored at 2-4° C in darkness for two and a half months to give them a new cold treatment (simulating winter). The plants were placed in

the greenhouse again 17 June 2010. For exposure dates and stages of development, see table 2.

At the different development stages, one fully emerged leaf from each of the 16 plants were induced with 124 kBq of $^{14}CO_2$ for 7 days in Styrene Acrylonitrile Copolymer (SAN) containers (22 cm x 33 cm, h 9 cm) from VITLAB sealed with oil grease (Statoil) to prevent the $^{14}CO_{2(g)}$ from escaping. The procedure for producing $^{14}CO_2$ was done by the following: Radioactive labelled $CaCO_3$ in aqueous solution, NEC-086S Sodium Bicarbonate, [^{14}C] – specific activity 310.8 MBq $mmol^{-1}$, were purchased from Perkin Elmer®. The closed container was sealed containing the plant leaf (still attached to the plant stem) and a glass-vial containing 3.2 ml 1,047 µCi/ml $Ca^{14}CO_{3(aq)}$. The vial inside the container was then added 1ml of H_2SO_4 through a 1mm hole at the top of the box to produce $^{14}CO_{2\,(g)}$ and the hole was sealed with oil grease.

Sample preparation for ^{14}C-determination

The plants were harvested after 7 days of $^{14}CO_2$ exposure, dried at room temperature for 4 hrs, packed in polyethylene bags and stored frozen (-20 °C) prior to desiccation. Plants were separated into root, rosette leaves, stem with leaves and inflorescence/flower before they were dried at 85 °C for a minimum of 24 hrs. The plant parts chosen for ^{14}C determination were weighed and a sample for ^{14}C determination (approx. 100 mg) was collected randomly from the plant part. The remaining plant parts were weighed (roots and shoots separately).

Oxidation of organically bound ^{14}C and fractional yield

The samples were incinerated to achieve complete combustion into water and CO_2 in a biological oxidizer (R.J. Harvey Biological Material Oxidizer OX 500) at an oxygen flow ~350 ml min^{-1} at 900 °C for 2 min. The combustion gas then passed a series of catalysts at 715 °C, before CO_2 was absorbed into a mixture of 460 ml toluene (Merck); 270 ml methanol (Merck); 270 phenylethylamin (Merck), and 5.5 g permablend III (Pacard). The ^{14}C containing mixture was directly transferred into counting vials, which were inserted into the liquid scintillation counter for measurements of the ^{14}C activity.

The oxidizer instrument was calibrated by determining the instrumental fractional yield. The fractional yield of ^{14}C for the oxidizing procedure was determined for each set of samples to be oxidized (different days). The fractional yield was determined (Eq. 1) by means of one replicate of blanks by burning paper (blank), one replicate of burned paper with 50 µl of a standard

containing (actual yield), and one replicate of burned paper then added 50 µl of standard (theoretical yield). The samples β-activity was corrected for the fractional yield.

Fractional yield (%) = [(actual yield – blank) / (theoretical yield – blank)] * 100 – (Eq. 1)

Liquid scintillation counter measurements

The β-radiation from ^{14}C ($E_{\beta max}$=156 keV) was measured using a Pacard Tri-carb 2900 TR liquid scintillation counter. Counting time was set to a maximum of 30 min or to a standard error of 2 %. Standard errors are based on counting statistics.

Data preparation

The results of β-radiation were given in disintegration per minute (DPM1), and were corrected for the fractional yield (Eq. 1). The corrected DPM1 was divided with 60 to calculate the amount of Becquerel (Bq) in each sample. By dividing the amount of Bq with the weight of the sample (Bq/g), and multiplying it with the weight of the plant part, it gave a calculated value of Bq found in the plant parts.

Residual ^{14}C (Bq) calculated from the plant parts measured (not including the exposed leaf) were defined as 100 %. The percentages were proportioned to the amounts and areas of the plants they were detected.

The plants were sorted into groups after development stage and number of cold treatments in order to investigate possible patterns in which way the carbohydrate reserves were transported.

2.6. Seed germination experiment

Seeds

Seeds of *B. orientalis* were collected from the roadside population at Mortensrud, Oslo four times during the summer 2010 (Table 3).

Table 3 Harvesting dates - seeds

Harvest	Date
1.	06.07.2010
2.	20.07.2010
3.	03.08.2010
4.	17.08.2010

Seeds were sampled from the five top spikes of the main shoot from ten different plants and dried at room temperature (20 °C) for seven days. The seeds were stored in paper bags in the dark at 2-4 °C.

Cold stratification

50 seed capsules from each of 4 harvest times and 2 treatments were placed on Petri dishes and made in four replicates, totally 32 dishes. The seed capsules were placed on two layer of filter paper wetted with distilled water (4 ml) in 90 mm diameter Pertri dishes. The dishes were sealed with parafilm and wrapped in aluminium foil before stored dark in a cooling room at 3 °C for 6 and 12 weeks.

After 6 weeks, 16 Pertri dishes were opened. 1.2 ml water was added to half of the dishes (2 from each harvesting date) resealed and placed in the climate chamber for 4 weeks.

After 12 weeks a new batch of 16 dishes were taken out and opened. The seeds were cleaned in tap water and rinsed in distilled water, due to fungi, before placed in new Petri dishes (90 mm) with two layers of filter paper wetted with 4 ml distilled water. The dishes were sealed with parafilm and placed in the climate chamber for 4 weeks.

Germination conditions

The seeds were given full-light treatment (50µmol/m^2/s) in a room with diurnally fluctuating temperature (25/10 °C). After 2 and 4 weeks, germinated seeds were counted and removed.

2.7. Statistical analysis

The results from the two field experiments, the isotope experiment and the greenhouse experiment were tested by GLM model by Minitab 16, Statistical Software, (Minitab Inc 2011). Differences between tested subjects are considered significant when $P \leq 0.05$. Bonferroni was used as multiple test (Minitab Inc 2011).

3. Results

3.1. Field experiment at Ås, transplanted plants

The mean rosette diameter increased throughout the experiment from 7 cm to 13 cm, in the same period the mean length of the longest leaf increased from 4 cm to 12 cm. Five out of twelve plants had more than nine unfolded leaves in the rosette in the beginning of May. In mid May and the beginning of June four plants had more than nine unfolded leaves. In the last harvest (16 June) six out of twelve plants had nine or more unfolded leaves in the rosette.

60 % of the plants had a height of 5 cm or less in the beginning of May, and the tallest plant was 10 cm. The average rosette height was 10 cm in mid May and in the beginning of June, in mid June this had increased to 14 cm. Three plants had developed flower stem in the two harvestings done in June. The average height of the stem was 51 cm in the beginning of June and 61 cm in the middle of June.

All plants were in their vegetative stage in the beginning of May 2010. In mid May, one out of twelve harvested plants had developed inflorescence, BBCH code 51. Three plants had developed flower buds (BBCH code 59) in the beginning of June, and three plants were flowering (BBCH code 65) in the middle of June. The seven plants that had generative growth were all from replicate 5 and 6, medium towards large and large. The BBCH codes are explained in appendix 1.

Dry weight (DW)

Although the average above ground biomass appeared to increase with time (Figure 3), there were no significant differences in the aerial plant parts DW among the harvesting dates (P= 0.141).

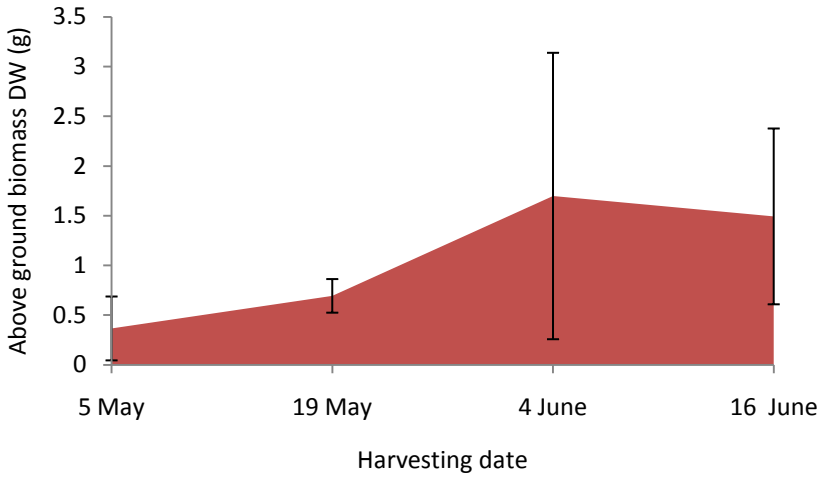

Figure 3 DW of aerial plant parts, n=12. The vertical lines represents SE (Standard error)

There were no trend towards decreased DW in the roots at any point (Figure 4) and no significant differences appeared in the roots DW among the harvesting dates (P=0.387).

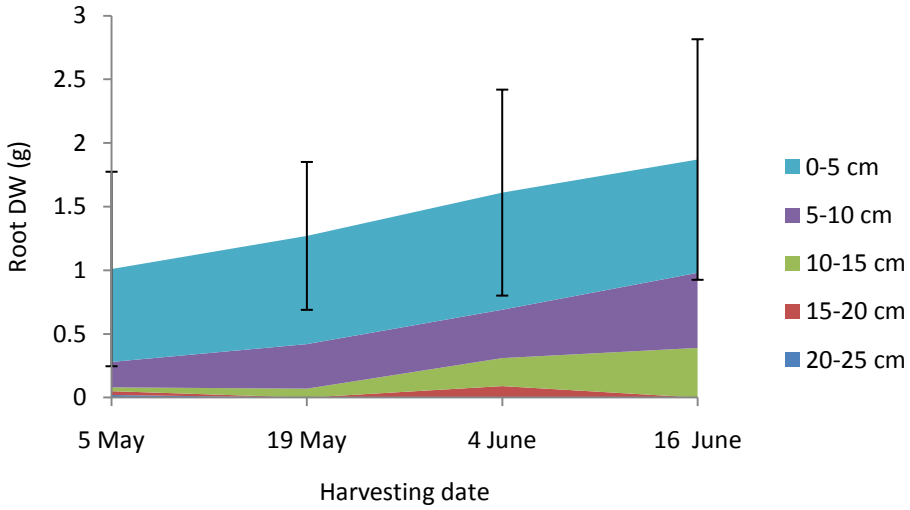

Figure 4 Root DW (n=6). Different colours represent root sections from different root positions. The top of the graph represents the average dry weight of one root at each harvesting date. The vertical lines represents SE

The roots fresh weight stayed more or less constant throughout the experiment, which made the dry weight percentage increase from 15 % - 28 % during the experimental period. The

average root length increased with 3.6 cm, from 12.1 - 15.7 cm in the experiment period. The average diameter of the root sections is given in table 4.

Table 4 Diameter of root sections in mm. Data from the first harvest is missing, and data from one root in the third harvest is missing

Harvest date/ Root section	19.05.2010 n=6	04.06.2010 n=5	16.06.2010 n=6
0-5 cm	13	7	16
5-10 cm	8	5	7
10-15 cm	4	4	5

Sprouting ability

Number of new aerial shoots from root sections harvested in the beginning of May until the beginning of June 2010 increased. Roots harvested 4 June 2010 had the highest amount of new shoots, and mainly from the second root section (5-10 cm), the blue area in figure 5. Number of new shoots diminished again for roots harvested 16 June (Figure 5). There were no significant differences in the number of aerial shoots among any of the harvesting dates (P= 0.330).

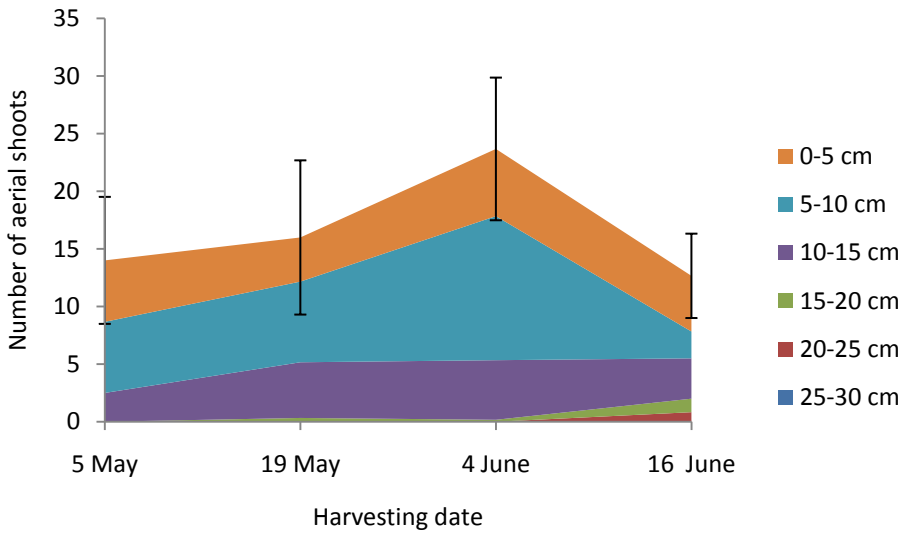

Figure 5 Number of aerial shoots from root sections after three weeks in the greenhouse. The top of the graph represents the average number of shoots from one plant at each harvesting date, n=6. Different colours represents different positions for the root sections.The vertical lines represents SE

The root sections produced new aerial shoots no matter where on the taproot they originally came from, but the low proportion of root sections from 15 cm and deeper has determined the outcome of the graph in figure 5. A complete table of the average root DW, fresh weight and number of new aerial shoots is given in appendix 3.

The shortest root section that managed to produce new shoots in this experiment was 1.4 cm long.

One root section from harvest three decayed, and one from harvest four partly decayed during the three weeks in the greenhouse.

3.2. Field experiment in Oslo, roadside population

The plants height increased until 22 June (Figure 6), when the plants were cut. At this point the height varied from 135 cm to 162 cm. The above ground biomass (Figure 7) is closely related to the plant height. In the beginning of August, the above ground biomass increased due to one big rosette plant with 10 cut off flower stems.

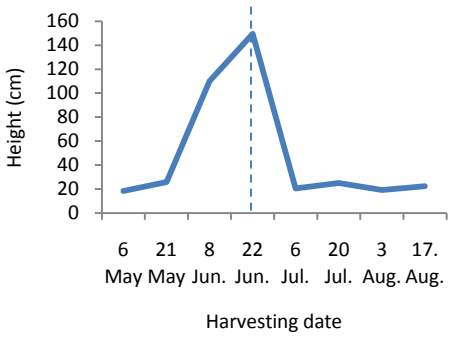

Figure 6 Plant height at harvest, n=6. The vertical line indicates when the population was cut.

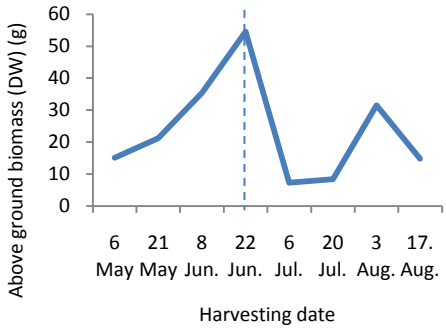

Figure 7 Above ground biomass (DW) at harvest, n=6. The vertical line indicates when the population was cut.

No plants had started generative growth in the beginning of May 2010. In the second half of May, the plants had started to elongate and inflorescence was visible (BBCH code 51). The flowers had started to open and some plants were in full flowering in the beginning of June (BBCH code 63 and 65). By the mid/end of June, all plants were flowering, and some had visible fruits (BBCH code 65, 67 and 69). See appendix 1 for BBCH codes.

No plants harvested after 22 June 2010 had any generative growth.

Sprouting ability

Significant differences in the sprouting ability and date of harvest were found among the second harvest (21 May) and the two harvesting dates in July (P=0,009). Root sections from one plant harvested 6 May 2010 produced 375 aerial shoots, of which 191 originated from the second root section (5-10 cm) (Figure 8). Roots harvested 21 May had a low ability to produce new shoots, on average, each root section only produced 1.13 new shoots at this point. The sprouting ability increased again for roots harvested in the beginning of June, and stayed more or less at the same level for the roots harvested two weeks later. Roots from plants harvested in July had greater sprouting ability than the ones harvested in June. The sprouting ability decreased for roots harvested in August (Figure 8).

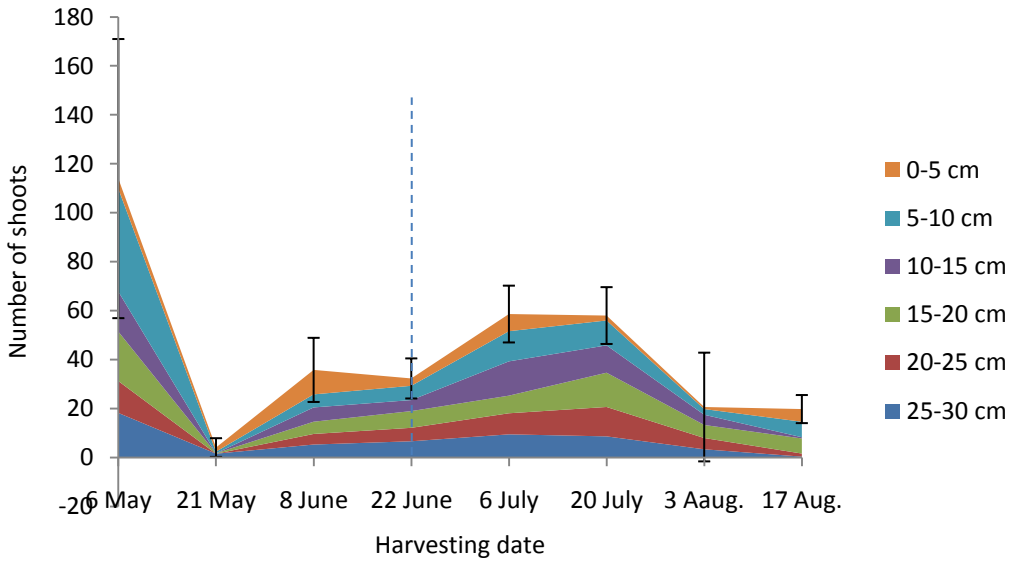

Figure 8 Number of shoots from root sections after three weeks in the greenhouse. The different colours represent the different positions on the taproots. The dotted vertical line indicates when the population was cut, and the solid vertical lines represents SE

Root sections developed aerial shoots regardless of their original position on the taproot. Figure 8 present the sprouting ability for root sections down to 30 cm. Because of the low proportion of roots longer than 30 cm, observations from 30 cm and further down are not

included in the figure. A complete table over the average number of shoots from root sections at different root positions and times of harvest is given in appendix 4.

A large proportion of the root sections harvested 21 May decayed or partly decayed during the three weeks in the greenhouse (Figure 9). The whole root of one plant completely decayed.

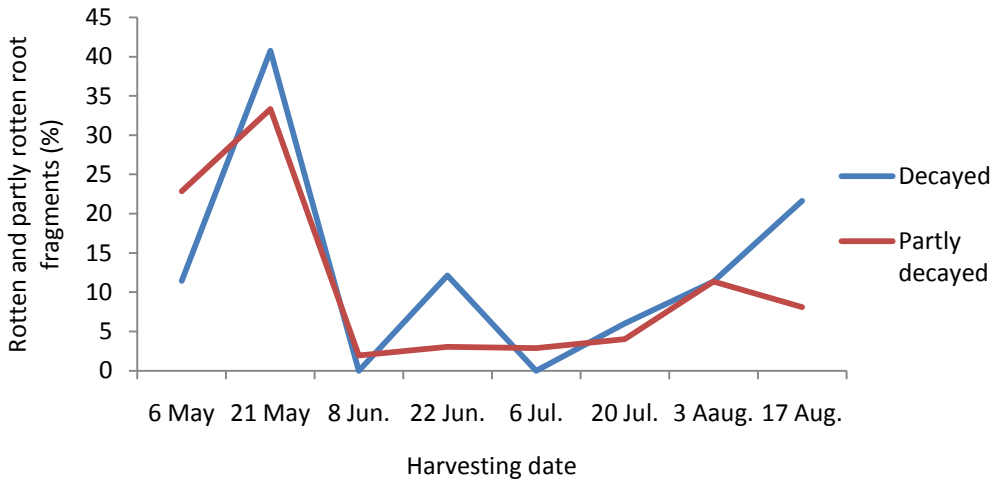

Figure 9 Proportion of decayed and partly decayed root sections from Oslo after three weeks in the greenhouse.

When the sprouting ability decreased, the proportion of decayed and partly decayed root sections increased, and vice versa (Figure 9). The proportion of partly decayed and completely decayed root sections decreased and increased simultaneously (Figure 9).

3.3. Isotope experiment

Plant and root characteristics

The root and aerial plant biomass increased with plant age throughout the experiment (Figure 10). From the first to the third ^{14}C exposure treatment (period before cold treatment), the shoots DW increased from 1.12 g to 18.97 g, whereas the shoots DW increased from 9.93 g to 14.17 g in the period after the cold treatment (Figure 10).

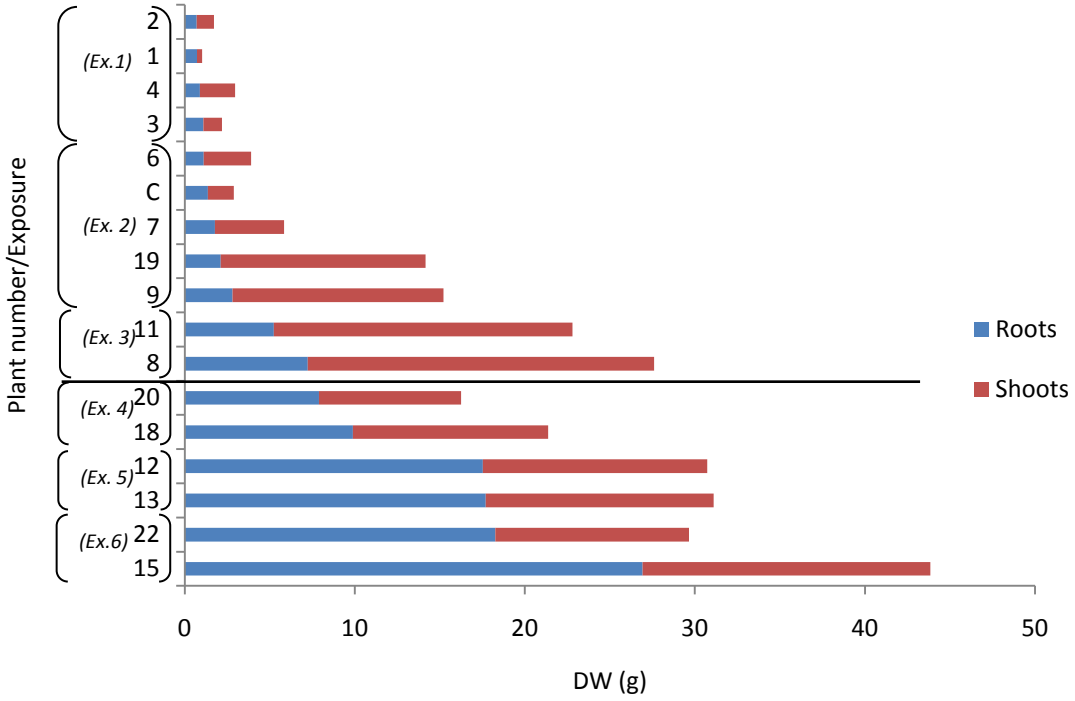

Figure 10 Root and above ground biomass of each study plant. The plants are listed after increasing root DW. The roots DW increased with age, the first exposed plants had the lowest DW and the last exposed plants the highest. Horizontal line between exposure 3 and 4 indicates time of the cold treatment. Exposure number is in brackets.

All the plants above ground plant parts are described in table 5. BBCH identifications keys are described in appendix 1.

Table 5 Characteristics of each plant's above ground plant parts

Plant No	Exp	Rosette height (cm)	Stem height (cm)	No of unfolded leaves - rosette	No of unfolded leaves - stem	Length of the longest leaf (cm)	Rosette diam. (cm)	BBCH code	Development stage
1	1	15		6		11	12,5		Small rosette
2	1	25		7		22	21		Small rosette
3	1	22		8		19	20		Small rosette
4	1	23	29	7	6	16	22	55	Elongation
6	2	28		17		24	39		Medium rosette
7	2	31		17		28	40		Medium rosette
C	2	26		8		22	39		Medium rosette
9	2	24	104	12	14	18	30	65	Flower
19	2	23	97	10	16	17	27,5	65	Flower
8	3	46		26		44	73,5		Large rosette
11	3	46		14		45	60		Large rosette
Cold treatment period									
18	4	27		60		22,5	34		Rosette
20	4	28		26		23	29	51	Rosette
12	5	29	67	17	14	25	35	65	Flower
13	5	32	50	24	12	27	29,5	63	Flower
15	6	27	24	47	7	21	40	55	Elongation
22	6	26	41	54	7	19,5	35	55	Elongation

Natural ^{14}C concentration and ambient level

The control plant contained 25.27 Bq ^{14}C with no difference between the root and the aerial plant parts. Natural occurrence of ^{14}C in the stem and flower was not determined. The ambient level of ^{14}C did not affect the percentage distribution of ^{14}C in the treated plants. The concentrations of residual ^{14}C in the exposed plants were therefore not corrected for the ambient level.

Concentration of ^{14}C in the exposed plants

The total recovery of ^{14}C measured in the plants of the total induced ^{14}C varied from 14 to 54 % with an average of 29 % (Table 6). The proportion of the total ^{14}C recovered in the plants measured in the exposed leaves varied from 10 to 84 % with an average of 56 % (Table 6).

Table 6 Total recovery of ^{14}C measured in the exposed plants and the amount found in the exposed leaves

Plant number	Total recovery of ^{14}C (Bq)	^{14}C in the exposed leaf (Bq)
1	61054	14845
2	42200	32497
3	41401	22709
4	66742	43133
6	36271	30618
7	45006	29997
9	34955	25499
19	35180	25638
8	36530	19356
11	29456	18326
18	28307	21547
20	34662	26188
12	20362	2085
13	17662	5427
15	25078	8165
22	23751	9932

Distribution of ^{14}C in the plants

The distribution of total plant ^{14}C (Bq) are presented in figure 11, 12 and 13, and for each plant in appendix 5. The small rosette plants had the majority of ^{14}C in the roots (Figure 11 and 12), and were significant different (Figure 11, P=0.041) from the medium rosette plants which distributed the ^{14}C equally between the root and shoot. The large rosette plants had the highest proportion of ^{14}C in the roots (Figure 11).

The concentration of ^{14}C in the roots were significant different between the rosette plants and the flowering plants among the generative plants as a whole (Figure 12 and 13, P=0.008).

The plant which had started to elongate after one cold treatment had the majority of ^{14}C in the stem and the stem leaves, while the flowering plants had more than 50 % of the ^{14}C located in the roots (Figure 12).

Plants treated twice with a cold period generally started generative growth (except one). Plants at the rosette stage had the majority of ^{14}C located in the root (Figure 13). Plants exposed during elongation had higher proportion of ^{14}C in the stem and flower, but still more than 50 % was located in the roots. It was only a small difference in the allocation of ^{14}C in the plants exposed at the development stages elongation and flowering.

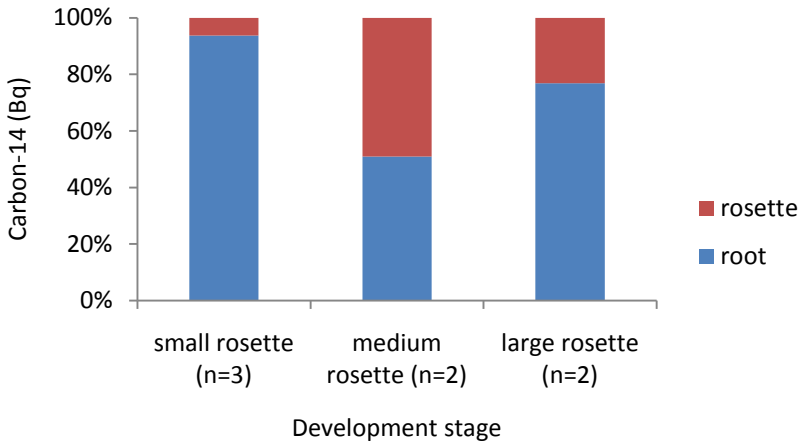

Figure 11 Distribution of ^{14}C in the exposed plants in percent. Vegetative growth after 1 cold treatment

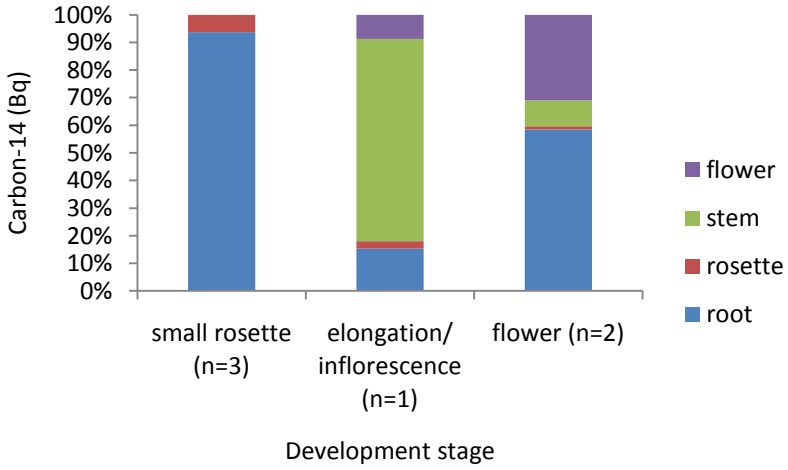

Figure 12 Distribution of ^{14}C in the exposed plants in percent. Generative growth after 1 cold treatment

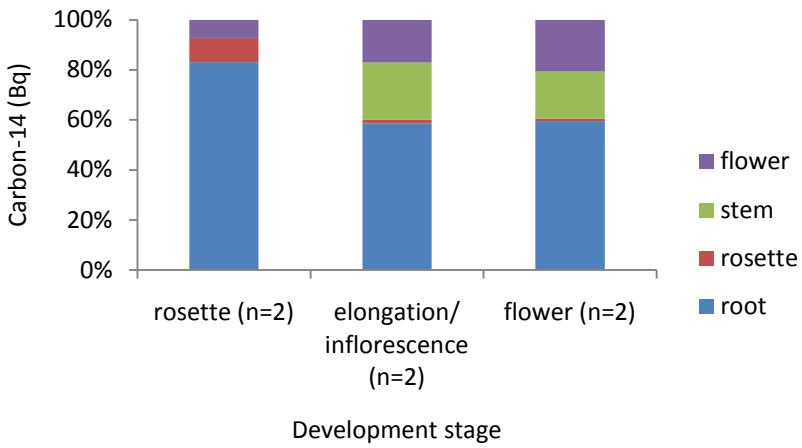

Figure 13 Distribution of ^{14}C in the exposed plants in percent. Generative growth after 2 cold treatments

3.4. Seed germination

Germination did not occur in any seeds after 6 weeks of cold stratification. There were considerable amounts of mould in the Petri dishes, and mainly on the fruits. Some fruits had begun to go soft and it was possible to open them with finger nails, some were still hard and impossible to open.

12 weeks of cold stratification resulted in 5 % germination of seeds harvested 20 July and 3 August following 2 weeks in the climate chamber. Following 4 weeks in the climate chamber the result were 18 % germination for seeds harvested 20 July and 6 % for seeds harvested 3 August 2010.

The highest germination rate was found for the seeds harvested 20 July, with 23 %, for seeds harvested 3 August, the rate was 11 %. Germination did not occur in any seeds harvested 6 July or 17 August 2010.

4. Discussion

Vascular plants are weaker and more prone to damaged at the time during the growth season when they have a minimum of energy stored in their below ground plant parts. Action taken at this point is more likely to cause damage to the plants than action taken at times when the plants have greater energy reserves. Traditionally, a root dry-weight minimum has been used as an indicator for when a plant has a minimum of energy stored in its below ground plant parts (e.g. Håkansson 1969 and Fykse 1986), called the compensation point (Håkansson 2003).

In young plants (1-2 years old) of *B. orientalis* no dry-weight minimum of the below ground plant parts was found at any specific development stage. This was the case both for the plants transplanted in the field and for those planted in pots and grown in the greenhouse for ^{14}C exposure. Such dry-weight minimum have however been found for young uncut plants (1 and 2 years of age) of another stationary perennial weed with taproot, *Rumex longifolius*, in which a drop in the root dry-weight was found at the stage where the plants had started to elongate and the stem had a length of 35-45 cm (Fykse 1986).

Although no dry-weight minimum was found for young *B. orientalis* plants, differences in the location of the induced ^{14}C in young plants (1-2 years old) at different development stages were shown. The stage where the smallest proportion of ^{14}C was located in the roots, might be the stage where the source-sink dynamic of carbohydrate reserves shifts from the below ground plant parts being the source to being the sink (Teasdale et al. 2007), and appear as a weak point in the species seasonal life cycle. At this stage the plants had started stem elongation and had an average height of 31 cm and inflorescence with the first individual flowers visible, but still closed. The vegetative plants had medium sized rosette with an average rosette height of 28 cm and an average diameter of 39 cm.

This development stage is similar to the findings in *Rumex longifolius* (Fykse 1986) and in *Artemisia vulgaris*, which had a drop in the root dry-weight at the stage where the plants had started stem elongation and had an average height of 35 cm (Oliver 2008).

Among the young plants transplanted in the field at Ås it was not possible to detect any fall in the roots capacity to sprout and produce new aerial shoots from buds on the root sections at any point. In general they produced less new shoots than the older plants from the location in Oslo, which may have been caused by stressed plants. On the other side, for the older plants

from the roadside population in Oslo a minimum of regenerative capacity in the root sections was found from plants harvested in middle of May. At this point the plants were elongating and had an average height of 26 cm (the shortest plant was 17 cm and the tallest 39 cm) and the inflorescence were visible.

As well as having a low regenerative capacity at this point, a large portion of the root sections decayed or partly decayed during the three weeks in the greenhouse. At first we believed the cause to be the water regime, but because none of the root sections from plants harvested in Ås 19 May had decayed or even partly decayed (they shared greenhouse and had the same water regime), it seemed unlikely. Weak plants and plant parts e.g. roots are more prone to be attacked by pest organisms like fungi and bacteria, so this may not have been related to the water regime or an unfortunate coincidence, but probably a result of weak roots.

The development stage where the roots regenerative capacity was at the lowest corresponds well with the development stage found in the isotope experiment where the highest proportion of ^{14}C was detected in the aerial plant parts.

Based on these results it is likely that a correlation exists between the compensation point and the vegetative regenerative capacity from adventitious buds on the taproot of *B. orientalis*, at least in older regenerative plants. Fykse (1974) found that there was a relationship between the dry-weight minimum in underground plant parts and the regenerative capacity both in the creeping perennial species *Sonchus arvensis* as well as in three dock species (*Rumex longifolius*; *R. obtusifolius; R. crispus*) (Fykse 1986).

After cutting down part of the population at Mortensrud in late June, none of the cut plants developed new flower stems. This was evident not only among the harvested plants, but also in the rest of the population. The sprouting ability was anticipated to decrease after cutting because the plants would have to use of the stored energy in order to produce new flower stems, but the plants remained as rosette plants and the presented result shows that the sprouting ability in fact increased after cutting. This is in contrast to the findings in docks (*Rumex* spp.) where the sprouting ability of root pieces decreased considerably after the plants had been cut, though only temporarily (Fykse 1986). The dock plants in the experiment of Fykse, however, was younger compared to *B. orientalis* in our study.

As earlier stated and also shown in these experiments, root fragments from the whole length of the taproot of *B. orientalis,* similar to *Taraxacum officinale,* are able to form aerial shoots (Korsmo 1954). In docks (*Rumex* spp.) on the other hand, the ability of forming shoots are mainly limited to the upper 5 cm of the root (Fykse 1986; Zaller 2004). In *B. orientalis*, also very short root fragments (Dietz et al. 1999b) and single parts of the root (cortex and stele) (Steinlein et al. 1996), have the ability to be the source of new plants. In the field experiment at Ås, one root section as short as 1.4 cm and with a diameter of 1 mm managed to produce an aerial shoot from a planting depth of 1 cm. In contrast to this, in a German experiment, Dietz et al. (1999b) found root sections of 1 cm planted at a depth of 5 cm or deeper unable to produce aerial shoots.

Based on the observations done in this thesis seeds in their early stage of ripening (beginning of July) do not manage to germinate. The seeds reached maturity in the second half of July. Consequently, if plants of *B. orientalis* is mowed from mid July or later at this location or other with similar climate, and the plant material is left on the soil surface, they may increase the soil seed bank of this species.

5. Conclusion

Lack of resources often restricts the weed control to only take place a few times (2-3 times) a year. Based on earlier findings (Dietz & Ullmann 1998; Dietz et al. 1999a; Steinlein et al. 1996) and the findings in this thesis, it may be better to leave established populations of *B. orientalis* undisturbed until there are enough resources to do a thorough job of eradicating the population. Cutting a population once, if done late enough to stop the plant from flowering again and early enough to prevent seed ripening will probability prevent the plant from generative reproduction (between late June and mid of July in this study). But the rosette leaves will absorb enough light to build a large energy reserve in the roots, which make the plant able to produce several vigorous stems with large clusters of flowers the following year, and the result is an even more viable plant.

Based on knowledge of other perennial weed species; frequent action, such as hand weeding, mowing or use of herbicides, taken at the time when the species is at its weakest (compensation point) will eventually exhaust and obstruct the root from producing new aerial shoots, and most likely is this also the case for *B. orientalis*. But, practical experiences as well as

knowledge from scientific experiments about this species clearly tell us that there is no quick and easy way of eradicating this species. It is therefore of great importance to monitor this species and to take action against new occurrences as soon as they are detected while the population still consists of a few young plants rather than a large number of established older plants in order to successfully eradicate the species.

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Appendix

Appendix 1 BBCH-identification keys of weed species

Appendix 2 Field map Ås

Appendix 3 Table of the average number of aerial shoots etc. - Ås

Appendix 4 Table of the average number of aerial shoots - Oslo

Appendix 5 Distribution of ^{14}C in the plants

Appendix 1

Weed species Hess et al., 1997

Phenological growth stages and BBCH-identification keys of weed species

D = Dicotyledons,
G = Gramineae,
M = Monocolyledons,
P = Perennial plants,
V = Development from vegetative parts or propagated organs.

No code letter is used if the description applies to all groups of plants.

Code		Description
Principal growth stage 5: Inflorescence emergence (main shoot) / heading		
51		Inflorescence or flower buds visible
	G	Beginning of heading
55		First individual flowers visible (still closed)
	G	Half of inflorescence emerged (middle of heading)
59		First flower petals visible (in petalled forms)
	G	Inflorescence fully emerged (end of heading)
Principal growth stage 6: Flowering (main shoot)		
60		First flowers open (sporadically)
61		Beginning of flowering: 10% of flowers open
63		30% of flowers open
65		Full flowering: 50% of flowers open, first petals may be fallen
67		Flowering finishing: majority of petals fallen or dry
69		End of flowering: fruit set visible
Principal growth stage 7: Development of fruit		
71		Fruits begin to develop
	G	Caryopsis watery ripe
79		Nearly all fruits have reached final size normal for the species and location
Principal growth stage 8: Ripening or maturity of fruit and seed		
81		Beginning of ripening or fruit coloration
89		Fully ripe
Principal growth stage 9: Senescence, beginning of dormancy		
97		End of leaf fall, plants or above ground parts dead or dormant;
	P, V	Plant resting or dormant

Appendix 2

Field map – Kirkejordet, Ås → N

Replicate 1		Replicate 3		Rep. 5		Rep. 5	Rep. 6	Rep. 4	Replicate 2	
X	-	-	-	X	X	X	X	X	X	X
X	-	X	-	X	-	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	-
-	-	X	-	-	X	X	X	-	X	X
-	X	-	X	-	-	X	X	X	-	-
X	-	X	-	-	X	X	X	X	X	X
-	X	-	-	X	X	X	X	X	-	X
X	X	X	-	X	X	X	X	X	X	-
-	X	X	X	X	-	X	X	X	X	X
X	-	X	-	X	X	X	X	X	X	X
-	-	-	X	X	X	X	X	X	X	X
-	X	X	-	X	X	X	-	X	-	X
-	X	X	X	-	X	X	X	X	X	X
X	X	X	-	X	-	X	X	X	X	-
-	X	-	X	X	-	X	X	-	X	-
-	X	X	X	-	X	X	X	X	X	-
X	-	X	-	X	-	X	X	X	X	-
X	X	-	-	X	-	-	-	X	-	-
-	X	X	X	X	X	X	X	X	X	X
X	-	X	-	X	-	X	X	X	X	X
-	X	X	-	X	X	-	X	-	-	-
X	X	-	-	X	X	X	X	-	X	-
-	X	X	X	X	X	X	X	X	X	X
-	X	X	X	-	-	-	-	X	X	X

ROAD

Appendix 3

The average number of aerial shoots, root dry weight (DW) (g) and root fresh weight (g) of root sections harvested at Ås in the period 5 May to 16 June 2010.

	Plant No:	1 – 12	13 – 24	25 – 36	37 – 48
	Date:	5 May	19 May	4 June	16 June
Root section	Harvest:	1	2	3	4
0-5 cm	Arial shoots: DW (g): Fresh weight (g):	5.33 0.73 3.75	3.83 0.85 3.99	5.83 0.92 3.87	4.83 0.89 3.37
5-10 cm	Arial shoots: DW (g): Fresh weight (g):	6.17 0.20 1.70	7 0.35 1.77	12.5 0.83 1.85	2.33 0.53 1.85
10-15 cm	Arial shoots: DW (g): Fresh weight (g):	2.5 0.03 0.9	4.83 0.07 0.87	5.17 0.22 0.81	3.5 0.39 1.2
15-20 cm	Arial shoots: DW (g): Fresh weight (g):	0 0.03 0.19	0.33 0.15	0.17 0.09 0.19	1.17 0.32
20-25 cm	Arial shoots: DW (g): Fresh weight (g):	0 0.02 0.06			0.83 0.01
25-30 cm	Arial shoots: DW (g): Fresh weight (g):				

Appendix 4

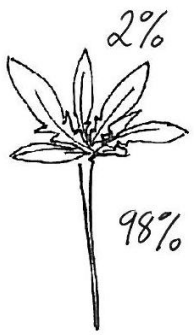
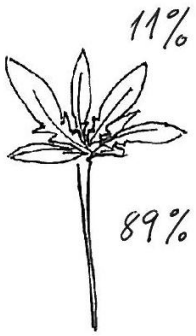
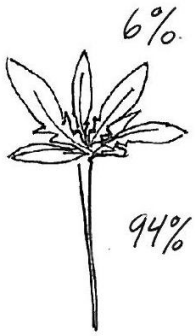
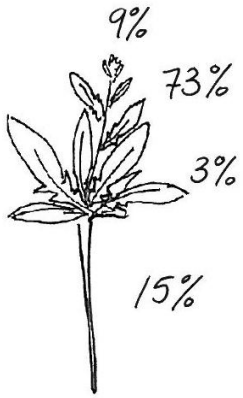
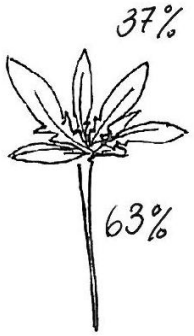
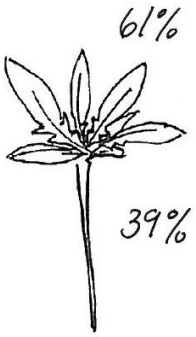
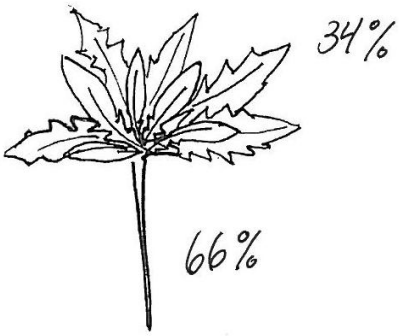
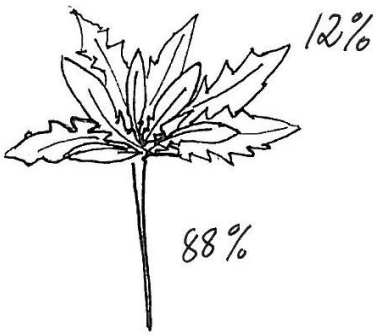
The average number of aerial shoots from root sections harvested in Oslo in the period 6 May to 17 August 2010

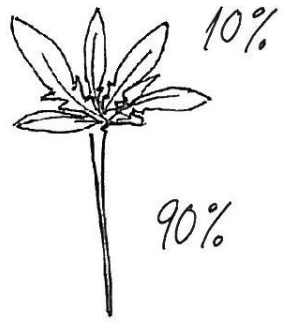
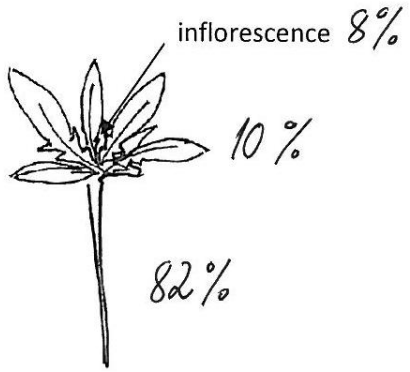
The number of observations is in ( ).

	Date:	6 May	21 May	8 June	22 June	6 July	20 July	3 August	17 August
Root section	Harvest	1	2	3	4	5	6	7	8
0-5 cm	Shoots	4 (6)	2 (6)	10 (6)	3 (6)	7 (6)	2 (6)	1 (6)	5 (6)
5-10 cm	Shoots	42 (6)	1 (6)	5 (6)	6 (6)	12 (6)	10 (6)	2 (6)	6 (6)
10-15 cm	Shoots	16 (6)	0 (6)	6 (6)	5 (6)	14 (6)	11 (6)	4 (6)	1 (6)
15-20 cm	Shoots	20 (5)	0 (6)	5 (6)	7 (6)	7 (5)	14 (6)	5 (6)	6 (6)
20-25 cm	Shoots	13 (5)	0 (6)	4 (6)	6 (4)	9 (5)	12 (6)	5 (5)	1 (5)
25-30 cm	Shoots	18 (4)	1 (6)	5 (6)	7 (3)	10 (4)	9 (6)	3 (5)	0 (5)
30-35 cm	Shoots	2 (3)	3 (6)	6 (5)	20 (1)	2 (2)	9 (5)	1 (5)	0 (2)
35-40 cm	Shoots		2 (5)	7 (4)	14 (1)	3 (1)	5 (3)	8 (3)	4 (1)
40-45 cm	Shoots		4 (3)	6 (3)			7 (3)	22 (1)	
45-50 cm	Shoots		0 (2)	5 (3)			7 (2)	28 (1)	
50-55 cm	Shoots		1 (1)	10 (1)			0 (1)		
55-60 cm	Shoots		0 (1)						

Appendix 5

Distribution of ^{14}C in the plants

Ex	One cold treatment			
1	1. Small rosette 	2. Small rosette 	3. Small rosette 	4. Stem elongation/ inflorescence 
2	6. Medium rosette 	7. Medium rosette 	9. Flower 	19. Flower 
3	8. Large rosette 		11. Large rosette 	

Ex	Two cold treatments	
4	18. Rosette 	20. Rosette/inflorescence 
5	12. Flower 	13. Flower 
6	15. Stem elongation/inflorescence 	22. Stem elongation/inflorescence