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Relationship between rhizome fragmentation, mowing and competition in control of common couch grass (*Elymus repens*)

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1 Preface

Finally my long and intricate journey to a master`s degree is completed. Thank God for the gift of stubbornness – with its advantages and disadvantages. It has been momentous and an honor for me to join researchers aiming to improve sustainability of agricultural practices. The master project has been a big piece of work and would have been unfeasible without the help of numerous people.

First of all, I would like to thank my supportive main supervisor at NMBU/ Bioforsk, Lars Olav Brandsæter, for contributing with his great knowledge and experience and an infectious inspiration. It has been an honor to work with the world`s worst weed and the world`s best supervisor. Further thanks go to my co-supervisors, Knut Asbjørn Solhaug at NMBU and Bo Melander at Aarhus University, Denmark, for their valuable comments and suggestions, often requested at short notice. Knut Asbjørn Solhaug deserves special thanks for his great patience with me in the laboratory.

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Dorothee Kolberg

2 Abstract

The perennial *Elymus repens* is considered to be one of the most important weeds on arable land in the Nordic countries. The 1st part of this thesis is principally a literature review on the species` biology and physiology related to control methods and its regrowth capacity, with emphasis on (storage) carbohydrates in plants. *Elymus repens'* high tolerance to control measures and its great regeneration ability makes it necessary to combine all possible control methods, direct and preventive, cultural and mechanical. Especially in reduced tillage all available measures might be practiced for optimal control of *E. repens*: use of competitive crops, mowing and potentially rhizome fragmentation. The 2nd part of the thesis is an independent scientific paper arisen from the experimental part of the master project where the relationship between (i) a simulated innovative belowground (BG) control method of rhizome fragmentation and the common aboveground (AG) control methods, (ii) mowing and (iii) competition (white clover), was investigated in a factorial pot experiment. *Elymus repens*` response to weed control was recorded in number of early and final shoots, AG and BG biomass and concentrations of simple sugars and fructan. Fragmentation showed a significant effect on some of the responses, but there was no clear linear relationship. Competition from white clover had a significant reducing effect on all responses. Decreasing stubble height had a decreasing effect on BG biomass and fructan concentration in BG plant material. Increasing cutting frequency had a decreasing effect on BG biomass, concentration of simple sugars in AG and of fructan in AG and BG plant material, but stimulated formation of new aerial shoots. The latter might be exploited in a starvation strategy combined with other control treatments. In comparison with reference pots, cutting seemed to be more important than stubble height or the developmental stage at cutting. The study reports several interactions between the different treatment factors and their levels, with essentially synergistic effect or potential to be exploited in a starvation strategy. Among those a new discovery was that high cutting frequency stimulated formation of new aerial shoots. The relative importance of the different factors in this study were competition, stage when cut, stubble height and fragmentation, ranking from high to low importance. In practical use the ranking might be different if different levels of competition are considered. Although the pot experiment may indicate that the innovative method of belowground fragmentation of *E. repens* has restricted control effect, this method might be more important if its effect is related to an undisturbed system lacking fragmentation totally.

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3 Introductory literature review and research context

According to the Weed Science Society of America weeds can be defined as ‘plants growing where they are not desired’. A member of a certain plant species might be considered to be a weed in one place, while it is not in some other. There can be different reasons for classifying a plant as a weed. In agriculture yield reduction is the main reason due to Naylor & Lutman (2002). Yield losses on account of competition by weeds are estimated to 10 % of total yield losses worldwide (Froud-Williams, 2002). Since agriculture started to change native vegetation 7000 years ago, farmers have tried to control weeds (Streibig & Jensen, 2009). This thesis takes a closer look at mechanical and cultural management of one of the species often considered as weeds: *Elymus repens*, commonly called couchgrass, common couch, twitch or quackgrass. Other scientific synonyms for that species are *Elytrigia repens*, *Agropyron repens, Agropyrum repens, Triticum repens*. Even though according to the definition above a species itself cannot be called a weed, it will for the sake of simplicity be referred to the species (*E. repens*) in this paper. *Elymus repens* is heavily distributed by (agri)culture in areas with temperate climate in a circumpolar way (Palmer & Sagar, 1963; Holm et al., 1977). It can be considered to be the most important weed on arable land in the Nordic countries, because of its specific adaptation to the northern climate (Palmer & Sagar, 1963; Håkansson, 1974; Holm et al., 1977; Salonen et al.; 2001). Korsmo et al. (1981) classified *E. repens* as creeping perennial with horizontal extension by rhizomes.

3.1 Elymus repens - biology and physiology

3.1.1 Regeneration and rhizome growth

Elymus repens regenerates by seeds and rhizomes. Usually 50 seeds per ear are produced. The seeds are viable for a relatively short time. But potential ecodormancy due to unfavourable environmental conditions for the seeds may last for many years when deeply buried in the soil. In general regeneration by seeding is of minor importance in *E. repens* (Palmer, 1958; Holm et al., 1977). It regenerates by ductile rhizomes creeping horizontally at a depth of down to 15 cm, in arable land mainly between 2 and 10 cm (Håkansson, 1968). The rhizomes are often heavily branched. At their nodes they form a few thin roots and buds for aerial shoots or branching. Further development of buds is depressed by apical dominance (Håkansson, 1974). In this type of dormancy the apical bud is dominating lateral buds and restricting them to grow. Fragmentation of the rhizome breaks the apical dominance and immediately leads to sprouting of adventitious buds (Brandsæter et al., 2010). Apical dominance has been described as being important for *E. repens*` success as a weed as it makes the rhizomes capable of fast regeneration after physical disturbance (Chancellor, 1974). Apical dominance is suggested to be result of competition between the apical bud and the lateral buds for water, nitrogen and soluble carbohydrates (McIntyre, 1969; 1970; 1971; 1972; 1981). In experiments where *Poa pratensis* rhizomes were decapitated under conditions with no light restrictions, growth was not restricted to one bud only. Buds closer to where the apex had been, started to grow and formed longer shoots than buds further away (Nyahoza, 1974). When the apex is removed from the rhizome or the rhizome is removed from the parent plant, restriction of lateral buds is released (Rogan & Smith, 1976). In rhizome fragments a new dominance pattern is formed and only one of the buds continues growing to an aerial shoot (Chancellor 1974; Nyahoza, 1974). The dominating bud is producing an aerial shoot, while the buds further away remain dormant or produce rhizomes (Rogan & Smith, 1976). Rogan & Smith (1976) suggested the parent plant to maintain apical dominance by continuously providing the rhizomes with gibberellin. When the rhizome is separated from the parent plant, the gibberellin supply and hence the dormancy is broken. Undisturbed rhizomes' tips spatially merge into aerial shoots as time passes. Rhizomes and vertical bases of aerial shoots possess great regrowth ability, so any below ground part of the plant is part of the vegetative reproduction system (stems), despite the thin roots which are responsible for nutrition and water supply (Håkansson, 1974).

3.1.2 Source-sink dynamics

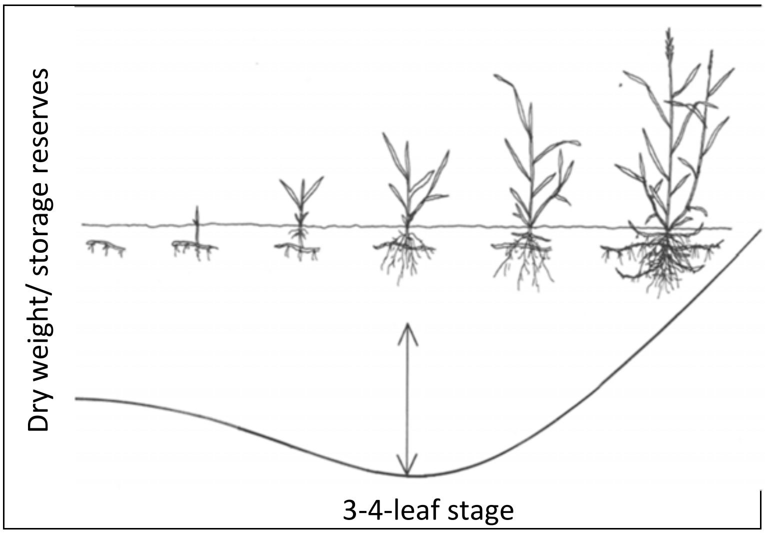

Figure 1: *Elymus repens* reaches compensation point at 3-4 leaf stage (Brandsæter et al., 2009)

During winter time most of the above ground parts in *E. repens* die. The first aerial shoots (primary shoots) develop from buds and rhizome tips during the first three weeks after ground frost has disappeared. This early growth is facilitated by carbohydrates reserves stored in the rhizomes (figure 2A). Under natural and undisturbed conditions *E. repens* reaches compensation point at 3 to 4 leaf stage (Håkansson, 1974). The compensation point in a plant`s development is the stage where the function of the belowground organs shifts from being a source to being a sink, regarding distribution of carbohydrate reserves (Håkansson, 2003). Compensation point is the weakest point in the plants` development, arriving at minimum dry weight (figure 1). At this point the plant is most vulnerable to any type of weed control. Plants of undisturbed *E. repens* usually reach 3 to 4 leaf stage during the first half of May in the Middle east of Sweden. It has been observed delayed compensation point, in terms of developmental stage, in dense stands with high competition. Competition can inhibit formation of new rhizomes and aerial shoots partially or totally (Håkansson, 1969b; 1974). At compensation point, formation of the side shoots starts (figure 2B).

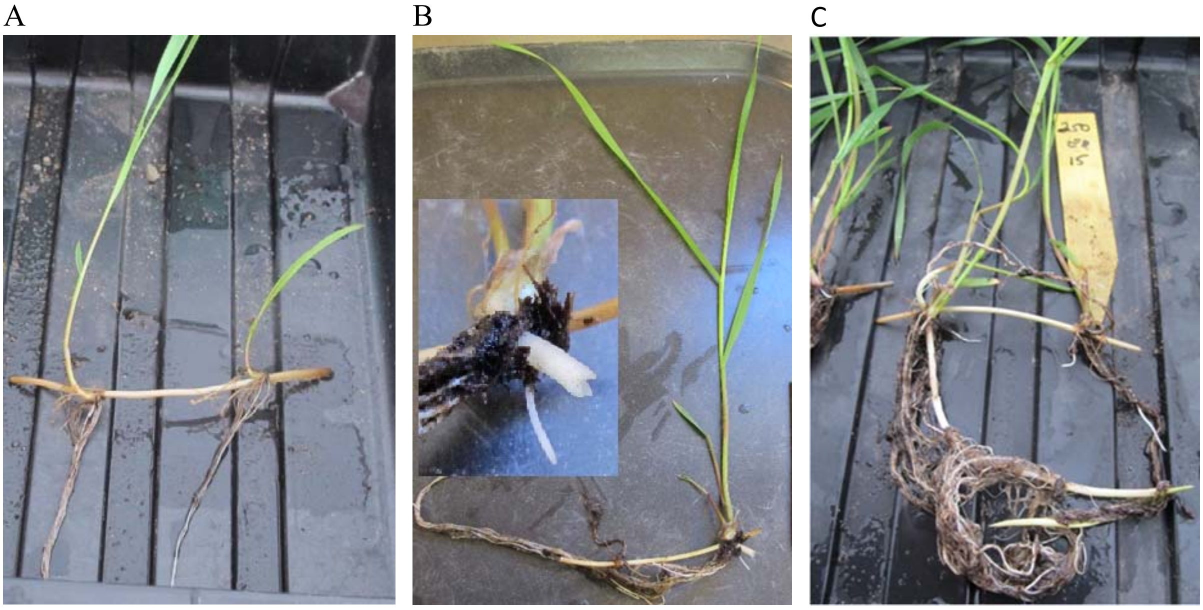

Figure 2: 2-leaf stage: *E. repens* approaching compensation point, rhizomes = source (A);
3-4-leaf stage: *E. repens* has just passed compensation point, initiation of new rhizomes (B);
5-6-leaf stage: new rhizomes visible in *E. repens*, rhizomes = sink (C), by Lars Olav Brandsæter, 2013.

Those side shoots can either form aerial shoots or new rhizomes. Under highly competitive conditions compensation point and the formation of side shoots can be delayed (Håkansson, 1974). After the plant has reached compensation point, photosynthetical supply of carbohydrates is greater than the demand for growth which is supporting photosynthesis and new rhizomes are formed (figure 2C).

3.1.3 Seasonal variations in growth, sprouting capacity and biomass

Many studies have concluded that the vegetative reproduction system of *E. repens* is not influenced by endodormancy (physiological rest) at any time of the year (figure 3), in contrast to other perennial weed species (Palmer, 1958; Håkansson, 1967; Håkansson, 2003; Brandsæter et al., 2010; Boström et al., 2013; Brandsæter et al., 2012), but there have been reported seasonal fluctuations in sprouting capacity and belowground biomass with a maximum during winter time (Leakey et al., 1977; Boström et al., 2013). The minimum ratio of shoot and rhizome biomass was found in October/ November. Boström et al. (2013) suggested that reduced photoperiod rather than declining temperature supported biomass allocation to rhizomes rather than to shoots in late

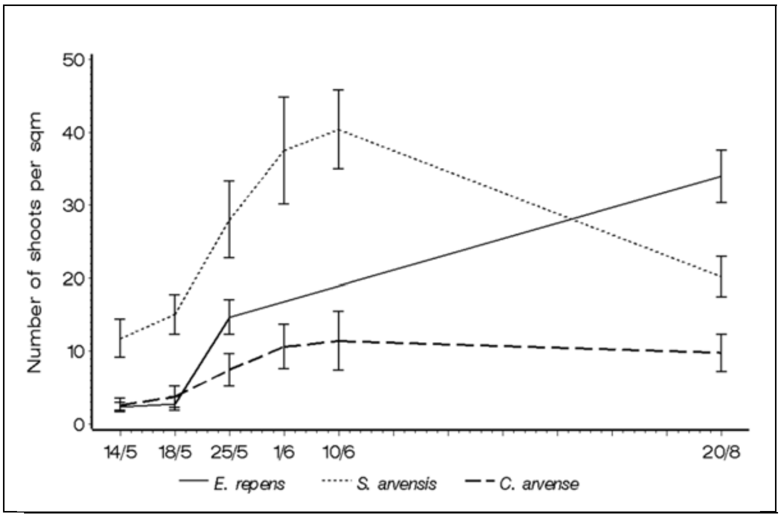

Figure 3: Seasonality in growth of perennial weed species *Sonchus arvensis*, *Cirsium arvense* and *Elymus repens,* from Brandsæter et al., 2012.

autumn. Accumulation of belowground biomass increased when conditions for photosynthesis diminished and aboveground growth was declining. When *E. repens* seedlings were transferred from long to short photoperiod, Williams (1971) found a decrease in growth in general, but an increase in rhizome growth. These seasonal fluctuations in sprouting capacity have been associated with concentrations of storage reserves in the rhizomes (Håkansson, 1967; Leakey et al., 1977). *Elymus repens* might be restricted by ecodormancy due to unfavorable climatic conditions. These conditions could be low temperature, lack of soil moisture, plant nutrients or short photoperiod. Regarding temperature ecodormancy is induced when average day temperature is dropping below 5/ 6 °C (Håkansson, 1974). Ecodormancy in *E. repens* arrives later in autumn compared to other perennial weeds (Brandsæter et al., 2006; Tørresen et al., 2010; Boström et al., 2013). That is why *E. repens* infestation can expand a lot in harvested cereal fields during autumn when there is no more competition from the cash crop (Håkansson, 1974; 2003).

The growth rate in *E. repens* is determined by temperature, soil moisture and light level. *Elymus repens* is a light-adapted species (Palmer, 1958). It prefers low temperature in combination with long photoperiod. That is the reason why this species is a better competitor and hence a more important weed in the Nordic countries compared to other parts of the world designated by temperate climate. Longer photoperiod increases *E. repens'* ability to form new rhizomes. In autumn shorter photoperiod redirects growth activity from formation of new rhizomes to growth of above ground plant parts and to a certain degree also to further growth of already existing rhizomes. Growth of existing rhizomes can proceed until late autumn only restricted by ecodormancy (Håkansson, 1974). Tørresen et al. (2007) found LT_{50} = -18 °C and -15 °C for *E. repens* from Northern and Southern Norwegian ecotype, respectively, lower than in other perennial weed species. *Elymus repens* has been reported to produce more aboveground biomass, but less rhizomes in number and weight under high nitrogen conditions compared to limited nitrogen supply. In contrast to that, buds at the low nitrogen rhizomes showed a lower sprouting ability than buds in high nitrogen rhizomes (Dexter, 1936).

3.2 Carbohydrates` role in resilience to control measures

3.2.1 Carbohydrates in plants

Carbohydrates in plants can be divided into structural and nonstructural carbohydrates. The structural ones are cellulose, non-cellulosic polymers (hemicellulose), pectic substances and lignin (Bailey, 1973; Halford et al., 2011). Total nonstructural carbohydrates (TNC) or the fraction of the nonstructural carbohydrates (NSC) is made up by simple sugars (fructose, glucose, sucrose and maltose), starch and fructan (Holt & Hilst, 1969; Smith, 1973; Fu et al., 1998). Nonstructural carbohydrates function as energy source, transport media, storage reserves (table 1) and signaling molecules (Halford et al., 2011). Water-soluble (WSC) carbohydrates are simple sugars and fructan (Longland & Byrd, 2006). The nonstructural fraction of carbohydrates in vegetative parts of grasses is commonly made up by monosaccharides glucose and fructose, disaccharides sucrose and maltose, and polysaccharides starch and fructan (table 1). In grass seeds also trisaccharides and stachyose are common. Seeds of *E. repens* contain high amounts of fructan (McLeod & McCorquodale, 1958).

Table 1: Most common nonstructural carbohydrates in vegetative parts of grass and their main functions (partially adapted from Smith, 1972; Lambers et al., 2008; Taiz & Zeiger, 2010; Duffus & Duffus, 1984; Offler, 1999; Halford et al., 2011)

		Function
Monosaccharides $C_6H_{12}O_6$	glucose	energy source, transport through cell wall, storage
	fructose	energy source, transport through cell wall, storage
Disaccharides $C_{12}H_{22}O_{11}$	sucrose	Transport in phloem sap, storage
	maltose	Sustained plant growth at night, transport through chloroplast membrane
Polysaccharides $(C_6H_{10}O_5)n$	Glucosans: Starches – glucose polymers	Storage in warm season C_4 grasses
	Fructosans: Fructans – fructose polymers	Storage in cold season C_3 grasses

Perennial grasses can be divided into two groups, starch accumulators and fructan accumulators. Tropical and subtropical C_4 species accumulate starch in their vegetative parts (Smith, 1972), while temperate C_3 species which are adapted to seasonal periods of dry or cold climate, the most important storage carbohydrates are fructan (Weinmann & Reinhold, 1946; Smith, 1972; Pontis, 1989; Chatterton et al., 1989; Halford et al., 2011). The cold season grass *Elymus repens* belongs to the tribe *Triticeae* (of subfamily *Pooideae*) which by Smouter & Simpson (1989) has been found to have the highest fructan concentrations among species of the grass family (*Gramineae* or *Poaceae*). Fructan metabolism should be regarded as a "selective advantage" because of its occurrence in evolutionary sophisticated plant families (Lewis, 1984). In fructan accumulating species, fructan is an additional storage carbohydrate, not just an alternative to starch (Chatterton et al., 1989; Nelson & Spollen, 1987; Pollock, 1986; Lewis, 1984).

3.2.2 Fructan structure

Fructan are linear or branched polymers of fructose, or polyfructosylsucrose, with a glucose unit at the end. There are five types of fructan: inulin, levan, mixed levan, inulin neoseries and levan neoseries, which differ in chain length, branching and fructosyl linkages (Halford et al., 2011). The names phlein and triticin are not in use any more (Lewis, 1993). Fructan in cold season grasses are of the levan or mixed type (Arni & Percival, 1951; Bonnett et al., 1997; Vijn & Smeekens, 1999). Levan (earlier called phlein in plants (Nelson & Spollen, 1987; Chatterton et al., 2006), is made up by β2-6-linked fructose polymers. The mixed type includes both β2-6 linked and β2-1-linked fructose polymers. In *E. repens* the fructan has been found to be heavily branched (Hammer & Morgenlie, 1990) in line with the classification as mixed type.

The degree of polymerization (DP) varies between species, but DP of up to some hundreds have been found in plants (Vijn & Smeekens, 1999; Bertrand et al. 2003; Chatterton et al., 2006). In cold-season grasses a high DP is found (Chatterton et al., 1989). There seems to be disagreement on categorization with regard to DP. Based on analysis of fructan in stem bases, Smith & Grotelueschen (1966) and Smith (1972) classified *E. repens* a short-chain fructosan accumulator, compared to the short chains in bromegrass stem bases (DP 26) and the long chains in timothy (DP 260). Vijn & Smekens (1999) define low DP as DP 3-5, while Bertrand et al. (2003) as DP 3. Suzuki & Nass (1988) used high DP for DP 7 or higher. Solhaug (1991) used the term short to describe DP3-10, long for DP>10.

3.2.3 Fructan storage - features and fluctuations

There are differences in fructan concentration between different plant parts. Highest fructan concentrations have been observed in the lower part of the stem in grasses and in belowground plant parts in general (Pontis, 1990; Solhaug, 1991; Slewinski, 2012). In grasses there are higher fructan concentrations in stem bases than in leaves (Waite & Boyd, 1953), and higher concentrations in leaf sheaths than in leaf blades (Smith, 1973).

Chapin et al. (1990) distinguished between different types of storage: “reserve storage” which competes with growth, “accumulation of storage” due to excess supply and “recycling”. For carbon storage the latter is of minor importance. The differentiation between reserve storage and accumulation of stores might be difficult in rhizomatous species. Allocation to existing reserve stores and accumulation in new stores may proceed at the same time.

Storage carbohydrates in general and fructan in grasses accumulate when environmental conditions are optimal for photosynthesis, but not for growth (Youngner, 1972; Pollock, 1984; Kinmonth-Schultz & Kim, 2011). In this case carbohydrate supply exceeds the demand for growth, allowing accumulation of storage. When stress conditions are ceasing, these reserves are used for growth again (Chapin et al., 1990). There can be different types of growth limiting and storage favoring conditions. Nutrient or water deficiency, high salt concentrations, high or low temperature, flooding/ oxygen deficiency, exposure to toxic compounds or UV-B radiation might be considered as plant stress (Archbold, 1938; Smith, 1973; Chapin et al., 1990; Halford et al., 2011). Also the morphological stage of the plant might influence the extent of storage accumulation. Plants in mature growth stage would rather store fructan in storage organs even though growth conditions are optimal (Smith, 1973).

Species adapted to high irradiance and low temperatures accumulate fructan (Pollock, 1986). Fructan accumulating species are able to continue photosynthesis and growth at temperatures down to 0 ° C (Pollock, 1986). In addition high DP fructan has been more closely related to cold hardiness than low DP (Suzuki & Nass, 1988). Cold-season species accumulate fructan in autumn when temperature is lower and days are shorter. Chatterton et al. (1986) found that low temperature favored fructan allocation for storage in *Agropyron ssp* and that this effect was more pronounced with shorter photoperiod. Solhaug (1991) got the same results for *Poa pratensis*. In *E. repens* sprouting capacity and belowground biomass is increasing when photoperiod is decreasing during autumn (Boström et al., 2013). As sprouting capacity has been associated with concentrations of storage reserves in *E. repens* rhizomes (Håkansson, 1967; Leakey et al., 1977) and photosynthesis is decreasing in autumn, the storage of fructan during autumn might compete with growth (Chapin et al., 1990). Accumulation of fructan is then not classified as accumulation of access photoassimilates, but must be classified as reserve storage. This agrees with observations of no development of new rhizomes *E. repens* in autumn. At that time of the year growth is restricted to old rhizomes (Håkansson, 1974).

As implied above the seasonal variations in fructan concentrations in grasses are connected to seasonal changes in growth rate (Chapin et al., 1990) and energy demand due to morphological changes in the plant. Fructan concentrations in grasses have been observed to be highest in late spring, decreasing at flower initiation and seed formation (Waite & Boyd, 1953). In rhizomatous grass species there has been observed increasing fructan concentrations during autumn (Chatterton et al., 1986; Solhaug, 1991). Bertrand et al. (2003) recorded stable fructan concentrations during winter in perennial forage species, while starch concentrations declined.

There have also been described diurnal variations in fructan concentration (Chatterton et al., 1989). Aboveground fructan concentration has been measured to be increasing during morning, with maximum values in the afternoon and decreasing during night (Holt & Hilst, 1969; Bowden et al., 1968; Longland & Byrd, 2006). Both seasonal and diurnal variations seem to be in line with the description of Chatterton et al. (1989) that cool temperatures imply high fructan concentrations.

Pollock et al. (1988), who found poor correlation between frost tolerance and fructan concentration in *Lolium perenne*, implied that fructan accumulation should be considered a consequence of less growth activity relative to photosynthesis. The mentioned results in *E. repens* and *P. pratensis* (Chatterton et al., 1986; Solhaug, 1991; Boström et al., 2013), connected to growth-competing reserve storage, are not in line with this approach. There seem to be two different approaches to the relationship between fructan and plant stress. It is discussed whether high fructan concentration should be regarded as a consequence of stress like low temperature, presented in the current chapter, or whether elevated fructan concentrations increase the plant`s tolerance against stress factors, described in the following chapter.

3.2.4 Fructan and stress tolerance in plants

Nonstructural carbohydrates in general and especially fructan has been associated with enhanced tolerance against abiotic stresses in plants (Vijn & Smeekens, 1999; Valluru & Van Den Ende, 2008; Livingston et al., 2009; Halford et al., 2011 and references herein). There are numerous publications about accumulation of NSC or WSC in relation to improved tolerance to low temperature (in crowns of *Secale cereale* (Fu et al., 1998), in *Poa annua* (Dionne et al., 2001), in transgenic tobacco (Li et al., 2007), in rhizomes of perennial *Sorghum spp*. (Washburn et al., 2013), in crowns of oat (Livingston & Henson, 1998)), to heat shock (in crowns of *Secale cereale* (Fu et al., 1998)), to flooding/ oxygen deficiency (in perennial ryegrass (Yu et al., 2012), timothy (*Phleum pratense* L.) (Bertrand et al., 2003), to drought, salt stress, nitrogen deficiency, exposure to selenium and exposure to UV-B radiation (reviewed in Halford et al., 2011).

In addition there are many papers reporting especially fructan accumulation in relation to improved tolerance to low temperature (in grasses (Pollock and Cairns, 1991), in crowns of *Poa annua* (Dionne et al., 2001), in transgenic *Lolium perenne* (Hisano et al., 2004), in transgenic tobacco (Li et al., 2007), in transgenic rice (Kawakami et al., 2008), in rhizomes of perennial *Sorghum spp*. (Washburn et al., 2013), in bases of cereals (Suzuki & Nass, 1988), in crowns of oat (Livingston & Henson, 1998)), to flooding/ oxygen deficiency (in perennial ryegrass (Yu et al., 2012), shoot bases in timothy (*Phleum pratense* L.) (Bertrand et al., 2003 and references herein)), to drought (in transgenic tobacco (Pilon-Smits et al., 1995)), to nitrogen deficiency (in rhizomes of *Phalaris arundinacea* (Kinmonth-Schultz & Kim, 2011)), to salt stress, heat shock, exposure to selenium and exposure to UV-B radiation (reviewed in Halford et al., 2011).

Most of the suggestions about fructan`s role in stress tolerance are based on correlative evidence. Not all physiological interrelations are investigated yet and the specific functions of fructan under different environmental conditions remain unclear. Fructan seems at least to play an indirect role in tolerance to various abiotic stress factors (Pollock, 1986; Hogg & Lieffers, 1991b) with an impact on long term persistence, overwintering and spring regrowth.

In agriculture also grazing or mowing constitutes stress for the plant. In addition, in *E. repens*` case, it might be exposed to stress through other mechanical disturbance by weed control measures.

3.2.5 Carbohydrate concentration and regrowth capacity

For description of biomass allocation in perennial plants the root/ shoot ratio is not adequate. The plant should at least be divided into leaves, stems and roots (Poorter & Nagel, 2000). In addition storage structures like rhizomes should be described separately (Kinmonth-Schultz & Kim, 2011). Ability to regenerate after disturbance, like agricultural activities, is a special feature for plants adapted to unstable habitats and based on their ability to utilize storage reserves (Klimes et al., 1993). During regrowth the plant needs to reallocate reserves to the storage organs in order to be prepared for the next stress period (Smith, 1973). In *Phalaris arundinacea* fructan reserves have been suggested to be an expression for competitiveness by supporting winter survival and early regrowth in spring (Kinmonth-Schultz & Kim, 2011). Carbohydrate concentration a certain time after cutting might therefore be regarded as a measure of the plant`s_potential to survive the next stress period, which might be further control treatment or winter time. If this potential exceeds the critical level for survival as suggested by May (1960), the reserves might rather be regarded as an expression of "growth not being made" under the prevailing conditions (Sheard, 1973) or the success of the control method implemented.

Some studies have reported missing relationship between carbohydrate concentration at the time of defoliation and shoot regrowth within autumn for perennial species (Hogg & Lieffers, 1991a; Hogg & Lieffers, 1991b). Hogg & Lieffers (1991b) reviewed those studies and suggested a rapid return to photosynthesis or dormancy to be possible reasons. There is a need to distinguish between regrowth capacity/ regrowth potential and actual regrowth/ regrowth ability. Actual regrowth is depending on regrowth potential (here expressed by reserve storage/ carbohydrate concentration), but not exclusively. Amongst other factors phenology and morphology of the plant might have an important impact on the actual regrowth (Trlica & Cook 1971; Smith, 1973). According to Smith (1973) concentration of storage reserves should be multiplied with the weight of the storage organ in correlation studies. Even the pool of reserves is not a valuable measure of regrowth ability as it does not take into account the plant`s ability to utilize the reserves. Despite of that, Chapin et al. (1990) implied that the pool size of storage reserves was an appropriate expression for the reserves contribution to growth potential. Also Teasdale et al. (2007) associated a plant`s carbohydrate concentration with its regrowth capacity, the potential for regrowth.

The differentiation between regrowth capacity and regrowth ability might explain the contradictory results from different studies. There has namely been found a good relationship between treatment effects and carbohydrate concentrations in the autumn following defoliation treatments in perennial species, which was suggested to be a consequence of proceeding respiration, restrictions in photosynthesis and allocation of reserves to regrowth (Trlica & Cook 1971). Hogg & Lieffers (1991b) described a good relationship between etiolated shoot regrowth and TNC reserves of *Calamagrostis canadensis*. Morvan-Bertrand et al. (1999b) reported a strong relationship between early regrowth and fructan concentration in the stubble at the time of defoliation in *Lolium perenne*. And Halling (1988) found a significant positive relationship between autumn TNC/ fructan concentration and relative growth rate in the subsequent spring in shoots and stem bases of timothy (*Phleum pretense*).

Regrowth after defoliation in grasses relies on stored reserves for only a few days under normal regrowth conditions. Bahrani et al. (1983) suggested that early regrowth in tall fescue (*Festuca arundinaceae*) was supported partially from reserves in addition to current photosynthesis. During regrowth, following defoliation at 4 cm, fructan reserves in the stubble of *Lolium perenne* were depleted rapidly, while reserves stored in the roots were relocated to growth to very little extend. After 3 days the plants already relied solely on photosynthesis again (Morvan-Bertrand et al., 1999a). If regrowth is hampered by additional weed control methods like unusual great depth or fragmentation, the critical level for survival would be at a higher level and stored reserves would be more important for regrowth. The time of compensation point, when the storage organs` role shifts from source to sink, is used as an indicator for susceptibility to mechanical weed control (Nkurunziza & Streibig, 2011).

3.3 Common management strategies

The most important cultural and mechanical methods for control of *E. repens* are the use of competitive crops, soil tillage and to some degree cutting. They might be used as direct methods to reduce or constrain an existing infestation or as indirect methods in order to prevent radiation.
Elymus repens' high tolerance to control measures and its great regeneration ability makes it necessary to combine all possible control methods. Non-chemical weed management should include both direct and preventive, cultural and mechanical measures (Melander et al., 2005). In organic agriculture it is of great importance to include weed control as part of the 'multifunctional crop rotation' (Olesen, 1999).

3.3.1 Competition

Arable crops' competitiveness is an important factor in weed control (Olesen et al., 2007) which could even be exploited to a larger extent (Weiner et al., 2001). Competition can be used as a preventive measure to constrain infestation of perennial weeds or as a direct control method combined with other methods (Håkansson, 2003; Goul Thomsen et al., 2011). Competition from a well-performing cereal crop, a dense sward, green fodder or cover crops, might be used to inhibit *E. repens,* due to its light sensitivity. Growth reduction caused by competition is greater in belowground than in aboveground plant parts. Less light weakens and can even eradicate *E. repens'* ability to form new rhizomes (Håkansson, 1974).
Brandsæter et al. (2012) did not find any significant effect of undersown red clover (*Trifolium pratense* L.) on *E. repens*, while Swedish experiments with red fescue undersown in winter wheat reduced *E. repens* by 40 % without any significant reduction in wheat yield (Bergkvist et al., 2010). The latter results supported Dyke & Barnard (1976) who obtained 29-88 % reduction in *E. repens* with broad red clover and 43-62 % reduction with ryegrass undersown in barley. But from these experiments it is important to know that rhizomes were buried at 20 cm depth, which is deeper than natural growth of *E. repens* and might have weakened its competitiveness. Goul Thomsen et al. (2011) found competition from green manure to be the most important factor in experiments combining different root length, burial depth, cutting and competition in *Cirsium arvense*. On the

other hand competition from cover crops is not as effective as stubble tillage and living mulch is incompatible with stubble cultivation in autumn if the living mulch is supposed to overwinter (Rasmussen et al., 2006).

3.3.2 Soil tillage

Different soil tillage strategies are used as direct control measure against existing *E. repens* infestation. These may include the use of various types of harrows or rotovators, terminated by ploughing. Such measures may either be performed (i) as bare fallow in connection to ordinary soil tillage prior to sawing, (ii) post-harvest as repeated stubble cultivation or in the most extreme case (iii) as bare fallow through a whole season. The latter is undesirable, due to economical loss, resource mismanagement and environmental disadvantages. Sustained intensive soil tillage as weed control strategy is only relevant in cases where infestation by perennial weeds is impossible to control by other means (Melander et al., 2012).

Fragmentation

Elymus repens is highly tolerant to soil tillage (Håkansson, 2003), because fragmentation of rhizomes by any type of soil tillage leads to release of apical dominance/ activation of buds and formation of new aerial shoots. The higher the grade of fragmentation, the higher the amount of aerial shoots (Permin, 1973). At the same time a higher grade of fragmentation has been found to decrease production of new rhizomes (Turner, 1968). This points to an increase in aboveground growth activity at the expense of belowground growth connected to severe grade of fragmentation. Because of *E. repens*` great tolerance to soil tillage, fragmentation needs to be combined with some measure which is starving out the rhizome fragments and limiting the available energy for the induced shoots (Brandsæter et al., 2012) – burial or defoliation at the plant`s compensation point. If soil tillage is terminated by deep ploughing, the aerial shoots` ability to reach the soil surface with available resources is reduced; if the fragmentation is combined with mowing of the aboveground plant parts before or at compensation point, the fragments will be depleted and their regenerative ability will be weakened (Permin, 1960; Håkansson, 1974).

Burial – ploughing depth

When fragmentation and burial are combined, the highest grade of fragmentation gives best effect of burial (Håkansson, 1968; 1974; Turner, 1968). In experiments a greater planting depth led to a lower production of new rhizomes (Turner, 1968; Håkansson, 1969a). Also in field experiments a depressing effect of ploughing with increasing ploughing depth has been reported in perennial weeds (Børresen & Njøs, 1994; Håkansson, 1974; Håkansson et al., 1998; Brandsæter et al., 2011). Deep burial (25 cm) of *E. repens* rhizomes led to 7% and 60% mortality after one growing season for 5 cm fragments and 15 cm fragments, respectively. None of the rhizomes survived the following winter (Fykse, 1983). In experiments with burial depth, fragmentation and defoliation shallow burial (10 cm) led to mortality of 28% of 7.5 cm fragments and of 54% of 2.5 cm fragments (Turner, 1968). Deeper burial decreased the relative amount of buds which formed shoots.

Shorter rhizome fragments went through a more severe depletion in reducing sugars (glucose + fructose) than longer ones. Concentration of reducing sugars and production of new rhizomes was less in fragments buried at greater depth (Turner, 1968). LeBaron & Fertig (1962) obtained an over 70 % reduction in fructan concentration in rhizomes of *E. repens* by ploughing. The reducing effect occurred early and rapidly, but diminished when no further control measures were conducted, along with regrowth of herbage. Bare fallow led to a 94 % reduction by the end of the season, which was lasting to the following spring.

Timing

In general it is necessary to take the biology of the specific perennial weed into account when timing mechanical weed management, because bud activity and shoot growth and hence the measures success might be regulated by dormancy (Brandsæter et al. 2010). Brandsæter et al. (2014) found that bare fallow with disc-based harrow can be an effective measure to control perennial weeds, but in *E. repens* the timing was of minor importance, due to absence of endodormancy, in contrast to other perennial weed species (Brandsæter et al., 2010). This is in line with Permin (1960), who found post-harvest soil tillage more effective against *E. repens* than other perennial weeds, and Brandsæter et al. (2006), Tørresen et al. (2010) and Boström et al., 2013 who described a late ecodormancy and absence of endodormancy in autumn compared to other perennial weeds.

Starvation vs dehydration

Stubble cultivation can be used in two different main strategies, depending on the climatic conditions: starvation or dehydration. Harrowing with e. g. disc coulters (figure 4A) is used to starve out the rhizomes under conditions which are favorable for resprouting of the injured rhizome fragments. The dehydration strategy involves rotating implement (figure 4B) which leaves the rhizomes on top of the soil and exposed them to sunlight and wind (Pedersen, 2010). The latter is a common method in Denmark. It has become more widespread also in Norway during the last years, although it previously has been a perception that the method is most successful in periods with dry and hot conditions (Brandsæter et al., 2009), which are scarce in Norway.

Figure 4: Stubble cultivation for control of *E. repens* by starvation - harrowing with disc coulters (A) or dehydration - harrowing with Kvik-Up (B), by Lars Olav Brandsæter, 2014.

3.3.3 Mowing

Repeated defoliation can be used to stimulate formation of new aerial shoots and deplete the rhizomes by impeding allocation of storage reserves. In *E. repens* this is effective throughout the growing season, due to lack of endodormancy, but less effective in autumn than earlier in the season (Dexter, 1936; Boström et al., 2013). Mowing has been shown to be most effective at high N-levels, because high nitrogen conditions support foliage growth and suppresses formation of new rhizomes (Dexter, 1936). Nyahoza et al. (1974) found a greater reduction in number of rhizomes produced in *Poa pratensis*, the greater the part of the aboveground herbage removed.

Mowing as part of combined control strategies

Brandsæter et al. (2012) concluded that mowing was a weak control measure compared to soil tillage, but valuable as part of a control strategy taking into account environmental aspects. Also Goul Thomsen et al. (2014) concluded that green manure with frequent cuttings is not as efficient as soil tillage in control of *E. repens*. Defoliation alone does not inhibit *E. repens* perceptibly, but is an important part of combined control methods (Håkansson, 1974), for example combined with post-harvest soil tillage in cereal production (Boström et al., 2013). According to *E. repens'* biology the optimum defoliation time is at compensation point, but this is often not in line with the optimal stubble height and frequency of the current crop. Another example for combined control methods is mowing together with competition from (green manure) ley or green fodder within a cropping system as part of a rotation (Goul Thomson et al., 2011). Teasdale et al. (2007) concluded "that continuous depletion of carbohydrates from the root system, resulting from a joint effect of mowing and competition by the cover crop, will decrease the regrowth capacity of … quackgrass". Brandsæter et al. (2012) did not recognize any interaction between mowing and competition. The latter was in contrast to Goul Thomsen et al. (2011) and Graglia et al. (2006) who found greater control effect of cutting in *Cirsium arvense* when the weed was exposed to competition. Turner (1968) obtained a stronger reducing effect on belowground biomass in *E. repens* with defoliation, compared to the effect of different levels of fragmentation and burial depth.

Mowing and carbohydrate concentrations

Turner (1968) observed the same reducing effect on concentration of reducing sugars. In general fructan allocation takes place when growth conditions are limited, but photosynthetical supply of assimilates is present. After defoliation regrowth is competing with fructan reserve storage and suggested to reduce fructan concentration (Pollock, 1984). Gräβler & Von Borstel (2005) described fluctuations in fructan concentration in aboveground biomass of a number of pasture grasses. During a growing season with 5 harvests fructan concentration was initially increasing, reaching a minimum at 3rd harvest and increasing above initial values at the last harvest on average for all examined grasses. *Elymus repens* as an exception did not show a distinct decline in fructan concentration after 3rd harvest in midseason.

In grasses in general, a lower stubble height or a higher frequency gives a lower weight of roots and/ or rhizomes (Harrison & Hodgson, 1939; Youngner, 1972). Stubble height and mowing frequency do express a complementary relationship (Youngner, 1972). *Elymus repens* and *Poa pratensis* are amongst the grasses with a better ability to adapt to severe defoliation than other grasses.

Stubble height

Rhizomatous species have a better ability to withstand defoliation at lower stubble height than other grass species (Harrison & Hodgson, 1939). Decreasing stubble height resulted in decrease in weight of rhizomes, roots and aboveground biomass of *E. repens* during a 7 weeks period with weekly defoliation treatment Harrison & Hodgson (1939). Brown (1943, cited in Youngner, 1972) reported decrease in rhizome weight with decreasing stubble height during summer following biweekly defoliation in *Poa pratensis*, but the opposite in the subsequent autumn. The reason for that might have been the use of rhizome reserves for production of new aerial shoots during summer, leading to the decrease in rhizome weight; followed by higher level of photosynthesis of a greater herbage pool and reallocation of reserves to rhizomes in autumn. May (1960) reviewed reserve carbohydrates to be the foundation of post-defoliation respiration in belowground plant parts and early aboveground regrowth.

Mowing frequency

Most effective control of *E. repens* has been obtained with short intervals (Dexter, 1936). Brandsæter et al. (2012) found a significant effect of mowing on number of shoots in *E. repens* in one of two experimental seasons. In their study, however, the cutting was only conducted one single time, in addition to the cutting effect of the combine harvester. Holmøy & Teslo (2000) found that two postharvest cuttings were more effective against weeds than one single cutting, even though not as effective as stubble tillage. With increasing frequency of defoliation, Turner (1968) obtained decreasing growth and biomass of new rhizomes in *E. repens*. With biweekly defoliation (4 harvests) new rhizome growth was depressed totally. In the rhizomatous weedy *Calamagrostis Canadensis*, native to North America, a single mowing led to increase in belowground carbohydrate reserves compared to unmown reference, while 4 times mowing (monthly) gave a reduction (Hogg & Lieffers, 1991a). Henskens (1993) found the same pattern for

E. repens. After a single defoliation, regrowth was found to be supported by photoassimilates originating from remaining herbage, while after repeated defoliation storage reserves from rhizomes were relocated to regrowth.

Timing

In experiments with different timing of the 2nd harvest Halling (1988) found little variation in simple sugars in shoots and stem bases of timothy when growth ceased in autumn, while there was great variation in fructan concentration, especially in stem bases. A later 2nd harvest gave less fructan in both shoots and stem bases in Central Sweden, but the opposite in Northern Sweden.

3.4 Topical challenges in weed control

Reduced tillage, also called “surface”, “minimum” or “conservation tillage” (Triplett & Worsham, 1986), has the potential to reduce the risk of erosion and loss of phosphorus to surface waters, to conserve soil structure, reduce greenhouse gas emissions, reduce energy demand and increase feasibility/ timeliness of agricultural activities (Riley et al., 1994; Holland, 2004; Jacobsen & Ørum, 2009; Morris et al, 2010; Tørresen et al., 2011; Bechmann et al., 2011). The great advantages in environmental and resource management of reduced tillage have led to its increasing incorporation in agriculture through governmental action in the European Union as well as in Norway (Landbruks- og Matdepartementet, 2011).

On the other hand reduced tillage leads to increasing weed pressure in general, and especially from grasses and perennial weeds like *E. repens* (Rydberg, 1992; Riley et al., 1994; Skuterud et al., 1996; Bond & Grundy, 2001 and references herein; Tørresen et al., 2003; Moonen & Barberi, 2004; Velykis & Satkus, 2006; Peigné et al., 2007; Melander et al., 2008) as traditional mechanical weed control is not an option. This might lead to enlarged use of herbicides (Riley et al., 1994; Tørresen et al., 2011; Melander et al., 2013 and references herein). During the last centuries use of herbicides already has increased dramatically (Håkansson, 2003). Today herbicides pose the main proportion of all chemicals applied in Norwegian agriculture, in majority glyphosate (Mattilsynet, 2011). Reported negative consequences of increased use of herbicides for human health and environment have led EU to take action by “establishing a framework for Community action to achieve the sustainable use of pesticides”. The directive stresses use of non-chemical methods, as used in organic agriculture or integrated pest management (IPM). After January 01 2014 all professional agriculture requires adaptation of the general principles of IPM (European Union, 2009). That means that use of mechanical and cultural control methods is emphasized, in contrast to the objective of reduced soil tillage. Because of increase in weed pressure reduced tillage is not common in organic agriculture in Europe (Peigné et al., 2007). Another future challenge is the anticipated increase in atmospheric carbon dioxide concentrations which has been found to stimulate *E. repens* growth and its tolerance to glyphosate (Ziska & Teasdale, 2000).

In order to cope with the weed challenge in organic as well as in conventional farming with reduced tillage there is a need for development of agricultural implement that controls perennial weeds without turning the soil. This is one of the objectives of the European collaborative project

"OSCAR - Optimizing Subsidiary Crop Applications in Rotations" (OSCAR Consortium, 2011, October 3). In conservation agriculture subsidiary crops might be used as cover crops, before or after the cash crop, or as living mulches in combination with the cash crop. Among several advantages of subsidiary crops they do have a competition effect against weeds. Part of the OSCAR project aims on control of perennial weeds while optimizing management of the subsidiary crops. In addition to the subsidiary crop`s and cash crop`s competition, the control methods included in the project are mowing and rhizome fragmentation done with novel implement. Experiments in Sweden and Norway (OSCAR Consortium, 2011, October 3) are described to include

> "differential fragmentation to trigger suicidal meristematic growth (bud sprouting) at deep soil positions. Cutting … rhizomes … will provide stimulation for buds to begin sprouting from under the cultivation depth and the intact deep root system will starve. The effect will be strengthened by the competitive effects of the CC when the shoots reach the soil surface. Furthermore shoots from the level above the cutting depth should be strongly stressed by frequent mowing. Therefore, stubble height is very important because this is directly proportional to the length achieved by the different weed species when they reach the compensation point ("weakest point")." (p. 35)

One of the new machines is a rhizome cutter with disc coulters used to fragment rhizomes and break apical dominance in *E. repens* without turning the soil. For the development of this new implement and the research related to that, the Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Århus University, University College of Hedmark, Kverneland ASA and the Swedish University of Agricultural Sciences are cooperating. The implement has the potential to optimize weed control in organic agriculture, leading to a higher yield and reducing environmental impact of tillage, and to reduce use of herbicides in conventional farming, reducing environmental impact and impact on human health. In the experimental part of this master project the details of this implement's potential effects on *E. repens* were studied by imitating its fragmentation effect combined with other control methods commonly applied in a cereal growing system.

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4 Scientific paper:

Relationship between rhizome fragmentation, shoot cutting and competition in control of *Elymus repens*

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4.1 Summary

With its extending rhizomes and great regrowth potential, the perennial *Elymus repens* is considered to be one of the most important weeds on arable land in the Nordic countries. In this study we investigated the relationship between (i) a simulated innovative belowground (BG) control method of rhizome fragmentation and the common aboveground (AG) control methods, (ii) mowing and (iii) competition (white clover), in a factorial pot experiment. *Elymus repens*` response to weed control was recorded in number of early and final shoots, AG and BG biomass and concentrations of simple sugars and fructan. Fragmentation showed a significant effect on some of the responses, number of primary and final shoots and AG biomass, but there was no clear linear relationship. Competition from white clover had a significant reducing effect on all responses. Decreasing stubble height had a decreasing effect on BG biomass and fructan concentration in BG plant material. Increasing cutting frequency had a decreasing effect on BG biomass, concentration of simple sugars in AG and of fructan in AG and BG plant material, but stimulated formation of new aerial shoots. The latter might be exploited in a starvation strategy combined with other control treatments. In comparison with reference pots, cutting seemed to be more important than the stubble height or the developmental stage at cutting. The study reports several interactions between the different treatment factors and their levels, with essentially synergistic effect. Exceptions were discussed as possible survival strategy in *E. repens*, which might be exploited in a starvation strategy, or due to choice of response to express regrowth capacity. Among those a new discovery was that high cutting frequency stimulated formation of new aerial shoots. The relative importance of the different factors in this study were competition, stage when cut, stubble height and fragmentation, ranking from high to low importance. In practical use the ranking might be different if different levels of competition are considered. Although our pot experiment may indicate that the innovative method of belowground fragmentation of *E. repens* has restricted control effect, this method might be more important if its effect is related to an undisturbed system lacking fragmentation totally. *Elymus repens'* high tolerance to control measures and its great regeneration ability makes it necessary to combine all possible control methods, direct and preventive, cultural and mechanical.

Keywords: couch grass, mechanical weed control, mowing, defoliation, cutting frequency, stubble height, developmental stage, regrowth capacity, fructan, reserve storage.

4.2 Introduction

Reduced tillage reduces the risk of erosion and loss of phosphorus to surface waters, conserves soil structure, reduces greenhouse gas emissions and energy demand and increases feasibility/ timeliness of agricultural activities (Triplett & Worsham, 1986; Riley et al., 1994; Holland, 2004; Jacobsen & Ørum, 2009; Morris et al, 2010; Tørresen et al., 2011; Bechmann et al., 2011). The great advantages in environmental and resource management of reduced tillage practices have led to its increasing incorporation in agriculture through governmental action in the European Union as well as in Norway (Landbruks- og Matdepartementet, 2011). On the other hand reduced tillage leads to increasing weed pressure in general, and especially from grasses and perennial weeds like *E. repens* (Riley et al., 1994; Bond & Grundy, 2001 and references herein; Tørresen et al., 2003; Moonen & Barberi, 2004; Velykis & Satkus, 2006; Peigné et al., 2007; Melander et al., 2008) as traditional mechanical weed control is not an option. This might lead to enlarged use of herbicides (Riley et al., 1994; Jacobsen & Ørum, 2009; Tørresen et al., 2011; Melander et al., 2013 and references herein). During the last centuries use of herbicides already has increased dramatically (Håkansson, 2003). Today herbicides pose the main proportion of all chemicals applied in Norwegian agriculture, in majority glyphosate (Mattilsynet, 2011). Reported negative consequences of increased use of herbicides for human health and environment have led EU to take action by "establishing a framework for Community action to achieve the sustainable use of pesticides". The directive stresses use of non-chemical methods, as used in organic agriculture or integrated pest management (IPM). After January 01 2014 all professional agriculture requires adaptation of the general principles of IPM (European Union, 2009). That means that use of mechanical and cultural control methods is emphasized, in contrast to the objective of reduced soil tillage.

The rhizomatous *Elymus repens* can be considered to be the most important weed on arable land in the Nordic countries, because of its specific adaptation to the northern climate (Håkansson, 1974; Salonen et al., 2001). It is especially resilient in agricultural systems with reduced tillage (Riley et al., 1994) where its most effective control measure, tillage, is not or only partially in use. There is a need for development of agricultural implement that controls perennial weeds like *E. repens* without turning the soil. Such an implement with disc coulters working at depths down to 15 cm is currently under development in Norway. It might be used e. g. in green manure leys during summer or in the autumn after harvest of cereals. By fragmenting the rhizomes, increasing the number of new aerial shoots and starving out the rhizomes it may improve the effect of AG weed control measures. In organic agriculture, this optimized weed control could potentially lead to higher yields and less use of stubble tillage, while it could reduce the need for herbicides in reduced tillage systems in general. The relationship between the implements' adjustment (grade of fragmentation) and other control measures, potentially applied in a cereal production system, needs to be investigated. Other mechanical and cultural control measures which are capable with reduced tillage might be competition from a cash crop or subsidiary crop (Håkansson, 2003; Goul Thomsen et al., 2011) or mowing (Turner, 1968; Håkansson, 1974; Graglia et al., 2006; Teasdale et al., 2007; Brandsæter et al., 2012; Boström et al., 2013; Goul Thomsen et al., 2014).

The objective of this study was to optimize control of *E. repens* by combining different levels of BG fragmentation with competition and different levels of mowing in a pot experiment, by imitating agronomical practices in a cereal growing system with reduced tillage. The research questions were: (I) What happens if the rhizomes are fragmented as desired? (II) How is a potential implements' adjustment (grade of fragmentation) related to stubble height, cutting frequency and competition? (III) How do the different methods and their levels influence each other?

4.3 Materials and methods

Belowground weed control: Rhizome fragmentation

Cutting of rhizomes with scissors.

Fragmentation with vertical rhizome cutter (Prototype by Kverneland ASA).

Aboveground weed control: Cutting of shoots

Aboveground cutting of *E. repens* with scissors.

Grass and straw chopper.

Competition: cover crop

White clover cover crop.

Clover undersown in cereals.

Figure 5: Treatments used in the simulating pot experiment (left) and their corresponding applied implements (right), by Lars Olav Brandsæter, 2013.

4.3.1 Study design, plant material and treatments

In a factorial outdoor pot experiment in 2012 and 2013 four different weed control treatments and their associated reference treatments were combined and replicated 3 times (figure 5, table 2):

Table 2: Treatment factors with levels and reference treatments included in the pot experiment

Treatment	Levels	Details/ reference treatments
(I) Competition	yes/no	by white clover cv. Milkanova (similar to field sow rates of 100 kg ha^{-1} in 2012 and 10 kg ha^{-1} in 2013)
(II) Degree of rhizome fragmentation	1x40, 2x20, 4x10, 8x5 cm rhizome length	
Cutting of shoots by scissors…		
…at different (III) stubble height	25, 50, 75 mm	Reference: no cutting
…at (IV) different developmental stages	2, 4, 6, 8 leaves	Reference: no cutting

Rhizomes of *E. repens* were collected from a highly infested area with cereal production in the vicinity of the experimental site the day before trial establishment. Initial branching was recorded, rhizome pieces were fragmented, weighed and buried at 5 cm depth in 10 l plastic pots filled with limed peat (Gartnerjord Tjerbo, Felleskjøpet), placed outdoors at Ås (59°40'N, 10°46'E). During the season the pots were watered and fertilized when necessary. Cutting treatments were conducted for 3 months (Medio July– Medio Oct 2012, Primo June– Primo Sep 2013) as many times as *E. repens* reached the predefined developmental stages.

4.3.2 Growth assessments and carbohydrate analyses

Timing of the cutting treatment at the predefined developmental stages was appointed by an arbitrary assessment of developmental stage on average for all *E. repens* shoots in the same group of the experimental factor stubble height, in terms of number of leaves per shoot. For comparison of this arbitrary assessment and the real developmental stage, number of shoots was recorded during the 2nd cutting of 2- leaf stage combined with the 1st cutting of 4- leaf stage and the 1st cuttings at 6-, and 8-leaf stage in 2012. Tillers were recorded as individual shoots. The first leaf of the main shoot was defined as part of the main shoot even after takeover by a tiller. Earlier cut leaves for 2nd cutting of 2-leaf stage were recorded together with new leaves. For investigation of the rhizomes' primary response to fragmentation, total number of shoots and number of main shoots per pot was recorded at 1st cutting of 4-leaf-stage: 24-29 days after establishment in 2012 and 19-21 days after establishment in 2013. At trial termination, when AG growth had ceased in 2012, two and a half months earlier in 2013, number of aerial shoots was recorded, AG and BG plant material was harvested and BG plant material separated into initial and new rhizomes. All plant material was dried in a drying cabinet for at least 2 days at 60 °C and weighed.

To explore degree of polymerization (DP) of the fructan present and feasibility of their separation from other carbohydrate species without underestimating the fructan portion in the case of low DP fructan present, thin layer chromatography (TLC) was performed prior to quantitative analyses. Extracts from above and belowground plant material from one mild treatment combination were selected, because high concentrations of fructan was expected. Water and ethanol extracts (25 and 50 µl per spot) were applied on Merck Silica gel 60 F254 aluminum plates with a layer thickness of 200 µm. The plates were developed twice in 1-butanol: isopropanol: water in the ratio 8:7:4 (v/v; Smouter & Simpson, 1989). Results were visualized by spraying with urea-phosphoric acid reagent, followed by heating at 105 °C for 10 min. Urea-phosphoric acid reagent was made from 100 ml 1M phosphoric acid in water-saturated butanol (butanol: water in the ratio 4:1, v/v), 3 g urea and about 5 ml ethyl alcohol (Wise et al., 1955).

Dried plant material from factorial combinations of lowest and highest treatment levels from 2013 and their associated reference pots were used for quantitative analysis of simple sugars and fructan in rhizomes and aboveground plant parts using anthrone method (Yemm & Willis, 1954). Dried plant material was ground with Tecator Cyclotec 1093 Sample Mill. Samples of 10-15 mg were extracted three times with 1.5 ml 90% ethanol (v/v, Smith & Grotelueschen, 1966) at 60 °C for about 30 min in a VWR Ultrasonic Cleaner with 3x 5 min sonication, shaken in between, and then extracted three times with 1.5 ml distilled water. Supernatants were combined for each sample after centrifugation (3 min, 15000 rpm). The extract, 0.02 ml (later 0.05 ml), was layered upon 2 ml ice-cold anthrone reagent together with 0.38 ml (later 0.35 ml) distilled water. Test tubes were shaken, placed in boiling water bath for 11 min, cooled on ice and absorbance was measured with spectrophotometer at 630 nm. Anthrone solution was made daily from 100 ml 70% sulphuric acid and 200 mg anthrone. Standards of sucrose and fructose with concentrations of 0, 50, 100 and 200 ml/l were used for analysis of simple sugars and fructan, respectively. The standards (0.4 ml) were analyzed together with the samples.

After anthrone method ethanol and water extracts from one aboveground sample were stored in the fridge. To explore the extraction method`s accuracy, two identical TLC plates of those saved extracts (25 µl per spot) were run and developed with the same procedure as above, but only one of them was visualized x1. An unknown substance appeared in the fructan region of the ethanol extract where no fructan would be expected, as fructan is not ethanol soluble. That is why the non-visualized TLC plate was used for further quantitative analysis. One cm bands of the crucial regions of the silica layer were scraped off the plate and eluted with 1 ml ethanol for the simple sugar and fructan regions of the ethanol extract and with 1 ml water for the fructan region of the water extract. Carbohydrate concentrations were determined using anthrone method. For judgment of the method of using pooled belowground plant material in the main carbohydrate analysis, initial and new belowground plant material from two treatment combinations, not included in the main analyses, were separately analyzed chemically. One mild and one tough treatment combination with 3 replications were picked out for that purpose (table 3). After mild treatment there were many new rhizomes and initial rhizomes were expected to be alive.

Table 3: Treatment combinations for mild and tough treatment in control of *E. repens* used for judgment of the pooled method

Treatment	Fragment	Stage	Stubble height	Competition
mild	20 cm	0	0	0
tough	5cm	2 leaves	50 mm	1

4.3.3 Data analyses and statistics

Statistical analyses and creation of boxplots were done by using stats package (version 3.0.1) in R. Conditional plots were created with lattice package (version 0.20-15). Reference treatments (table 2) were excluded from all statistical analyses, except for the primary response. Analyses took into account 4 control variables which were supposed to have an impact on the responses, but were of no scientific interest: variations in year, replication (/block), initial fresh weight of buried rhizomes and branching (present or not). Years differed in quality of initial plant material, climatic conditions, time of the season and experimental duration. Replications differed in time of establishment and hence development when treatment took place, and maybe in quality of initial plant material. With observations from only 2 years one could not draw any conclusions about the whole population of years or replications. That is why year and replication were treated as fixed.

Replication was treated as nested under year. Due to poor initial plant material, rhizomes were branched in a few cases in 2012.

For all models all possible interactions between the treatment variables were included initially, selection was performed with an error level of $\alpha = 0.05$ for interactions and $\alpha = 0.5$ for control variables (year, replication, initial fresh weight, branching), treatment variables (fragmentation, competition, developmental stage when cut and stubble height) were kept in any case. Exceptions: in primary response $\alpha = 0.05$ was used for control variables, too; in quantitative carbohydrate analyses control variable branching was excluded, because no branching was present in the chosen samples; in AG biomass, developmental stage when cut and stubble height were interpreted as control variables. All reported significant effects were based on $\alpha = 0.05$.

Real numbers of leaves per shoot at cutting were averaged for each developmental stage and compared to preceding arbitrary assessment.

Primary response

For rhizomes' primary response to fragmentation in terms of total number of shoots and number of main shoots per pot linear models were fit. Model selection was based on *P*-values from ANCOVA (Analysis of Covariance, including continuous covariate) F-test. Tukey Honestly Significant Differences test was conducted.

Final shoots and biomass

Zero values in shoots and biomass, due to failed performance (9 in 2012, 1 in 2013), were (defined as observations with "shoots==0 & aboveground biomass==0 & belowground biomass==0") excluded from the dataset. In addition there were some left censored zero values (5 in aboveground, 7 in belowground biomass in 2012), which represented positive values that were too small to be detectable. Therefore a very small value (0.005) was added to the responses in general. Generalized linear models were fit with error family Poisson (log-link) and Quasipoisson (log-link) for number of shoots per pot, with error family gamma (log-link, Faraway, 2006) and log-normal/Gaussian (identity-link) for AG and BG biomass. Model selection was based on ANCOVA (Chi -square) *P*-values with Df(Degree of freedom)-approximation by Satterthwaite, dispersion parameter (residual deviance/ residual degrees of freedom) and, for biomass, the difference between null deviance and residual deviance.

Carbohydrate analyses

One negative value in carbohydrate concentration, due to inaccuracy of spectrophotometer for absorbance measurements close to zero, was replaced by zero in the dataset prior to statistical analysis. Model selection was based on *P*-values from ANCOVA F-test.

For the judgment of use of pooled belowground plant material, Wilcoxon rank sum test was conducted to test for significant differences in carbohydrate concentration between mild and tough treatment and between initial and new rhizomes.

Attained control

Reference treatments were used to calculate %-wise degree of attained control of *E. repens*, in terms of reduction in number of shoots, AG and BG biomass, and degree of reduction in carbohydrates. For that purpose the variables which were found to have significant effect on the responses were explored graphically in comparison with the reference treatments without cutting; except for AG biomass, where the least significant variable (fragmentation) was excluded for practical reasons.

4.4 Results

4.4.1 Primary response to fragmentation and competition

Table 4: Degrees of freedom (Df) and *P*-values from ANCOVA for total number of primary shoots and number of primary main shoots (n=456), control variables, experimental factors, significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.

	Df	*P*	
		Number of primary shoots	
		Total	Main
Year	1	5e-14 ***	< 2e-16 ***
Replication	4	6e-07 ***	0.016 *
Fragmentation	3	0.177	0.002 **
Competition	1	0.001 ***	0.036 *

The highest degree of fragmentation (5x8 cm rhizomes) resulted in a higher number of primary main shoots than the two lowest ones (table 4, figure 6A). There was a significant lower number of primary main shoots and total number of primary shoots in *E. repens* in competition with white clover than without competition (table 4, figure 6B and C).

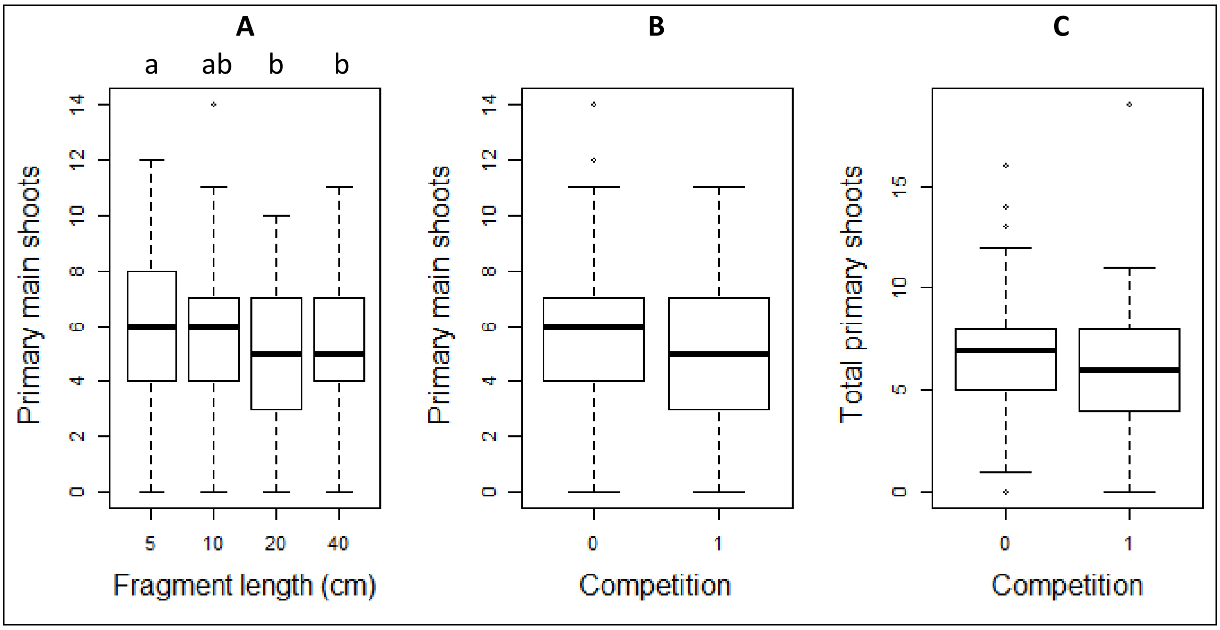

Figure 6: Number of primary main shoots at different initial degree of fragmentation (1 fragment = 1x40cm, 2 fragments = 2x20cm, 4 fragments = 4x10cm, 8 fragments = 8x5cm) of buried *E. repens* rhizomes (A). Number of primary main shoots (B) and total number of primary shoots (C) with (1) and without (0) competition. Values significantly different according to Tukey Honestly Significant Differences Test assigned different letter (n = 114 in A and n= 228 in B and C).

4.4.2 Final shoots and biomass

Table 5: Degrees of freedom (Df) and *P*-values from ANCOVA for number of shoots (n = 614), aboveground (AG) and belowground (BG) biomass (n =566) of *E. repens* at trial termination, control variables, experimental factors, (-) for interaction not included in the model, significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.

	Df	*P*		
		Number of shoots	AG biomass	BG biomass
Year	1	< 2e-16 ***	< 2e-16 ***	< 2e-16 ***
Replication	4	1e-13 ***	5e-05 ***	5e-06 ***
Initial fresh weight	1	0.136	0.362	0.003 **
Branching	1	0.001 **	0.013 *	0.038 *
Fragmentation	3	8e-06 ***	0.045 *	0.935
Stage when cut	3	7e-05 ***	< 2e-16 ***	< 2e-16 ***
Stubble height	2	0.420	< 2e-16 ***	2e-12 ***
Competition	1	< 2e-16 ***	< 2e-16 ***	< 2e-16 ***
Stage:competition	3	7e-06 ***	(-)	6e-07 ***

Number of shoots

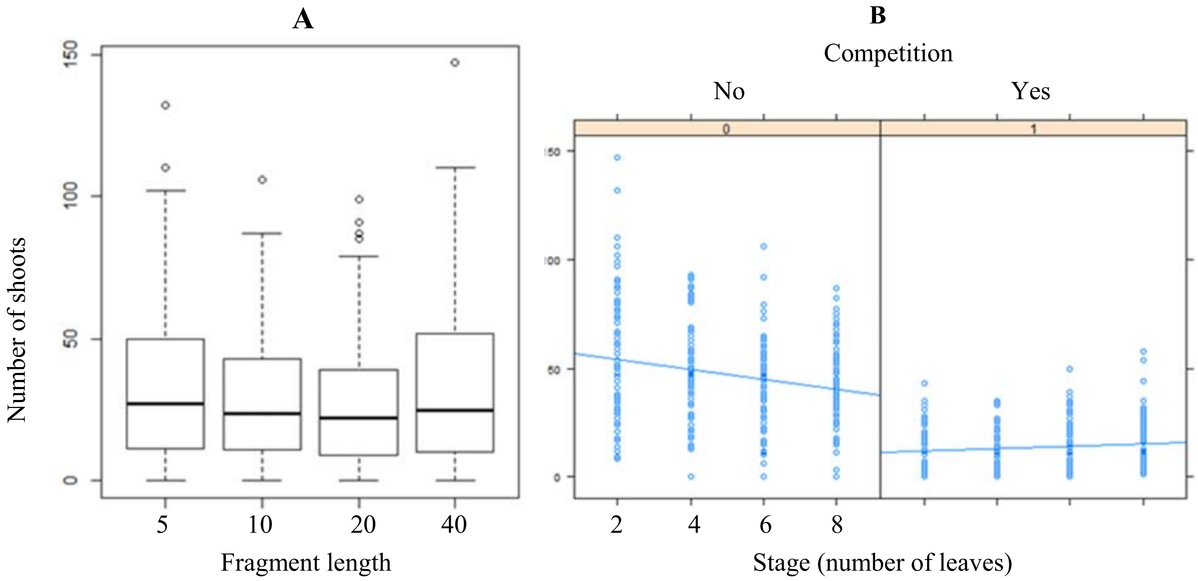

Figure 7: Number of *E. repens* shoots at trial termination after different initial degree of fragmentation (8x5cm, 4x10cm, 2x20cm, 1x40cm) of buried rhizomes (A); Interaction between developmental stage when cut (2-, 4-, 6-, 8-leaf stage) and competition (yes or no) for number of *E. repens* shoots at trial termination (B); (n = 141/142).

A higher degree of fragmentation led to a higher number of shoots at trial termination, except the highest degree of fragmentation which led to almost as many shoots as the lowest one (figure 7A). There was no effect of stubble height on number of shoots. Number of shoots increased with increasing cutting frequency (decreasing developmental stage) when competition was absent, while it decreased slightly/ almost was not affected by developmental stage when cut when competition was present, as shown by the significant interaction between developmental stage when cut and competition (table 5, figure 7B). Competition reduced number of shoots regardless developmental stage (figure 7B).

The visible deviation from the mean in the interaction plots is due to treatment effects (192 different combinations) in addition to experimental error and control variables (replications, fresh weight, year, branching).

Aboveground biomass

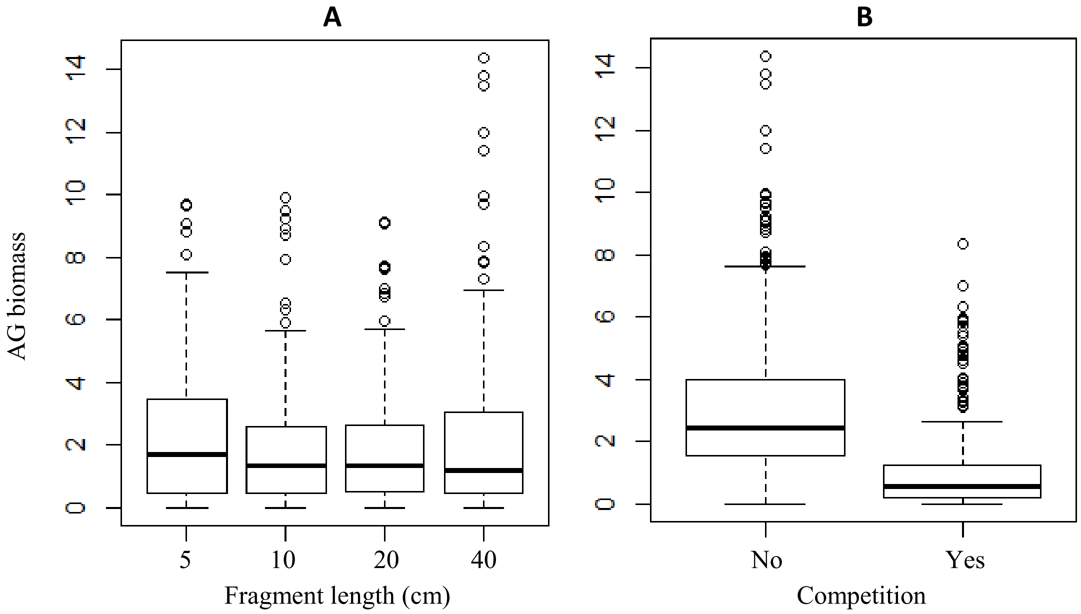

Figure 8: AG biomass at trial termination after different initial degree of fragmentation (8x5cm, 4x10cm, 2x20cm, 1x40cm) of buried rhizomes (A), with and without competition (B) (n = 141/142 in A, n = 283 in B).

There was no linear relationship between the significant effect of fragmentation (table 5) and AG biomass: There was slightly increasing AG biomass with increasing degree of fragmentation/ decreasing fragment length, except fragment length of 20 cm, which led to slightly higher AG biomass than 10 cm fragmentation (figure 8A). Competition led to a lower AG biomass (figure 8B).

Belowground biomass

There was no effect of fragmentation on BG biomass. Decreasing stubble height led to a decrease in BG biomass (figure 9A). Decreasing developmental stage gave a distinct decrease in BG biomass when competition was absent, while the decrease was less pronounced when competition was present, as shown by the significant interaction between competition and developmental stage when cut (table 5, figure 9B). Competition led to a lower BG biomass regardless developmental stage (figure 9B). Earlier developmental stage when cut/ higher cutting frequency led to lower BG biomass regardless competition present or not (figure 9B).

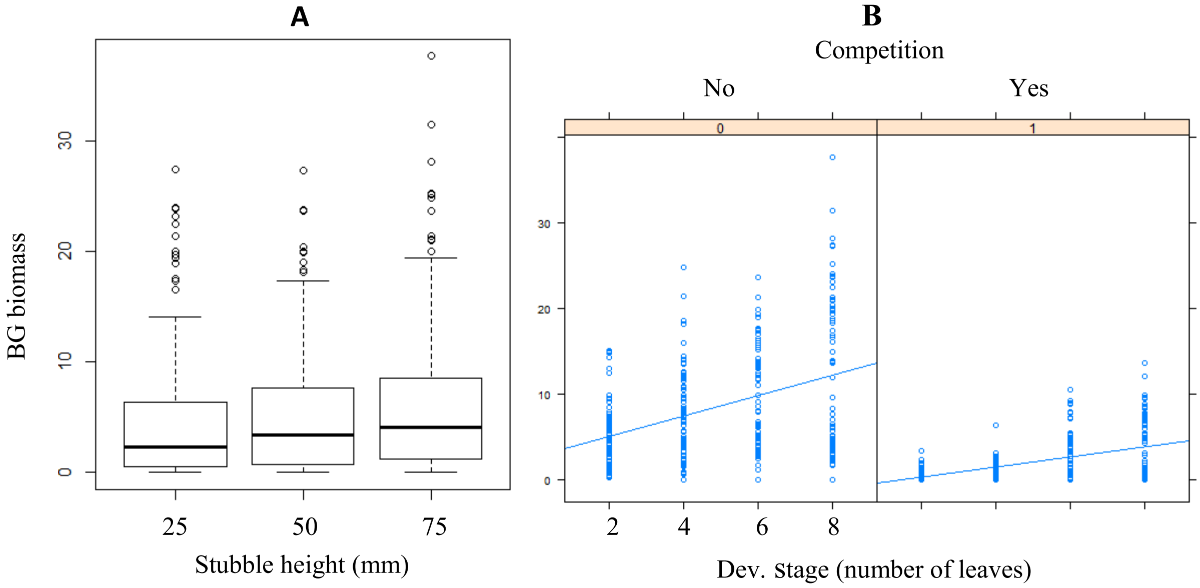

Figure 9: BG biomass of *E. repens* at trial termination after cutting at different stubble height (25, 50, 75 mm) (A); Interaction between developmental stage when cut (2-, 4-, 6-, 8-leaf stage) and competition (yes or no) in BG biomass of *E. repens* at trial termination (B); (n = 188/189 in A and 141/142 in B).

Control attained by cutting, compared to reference

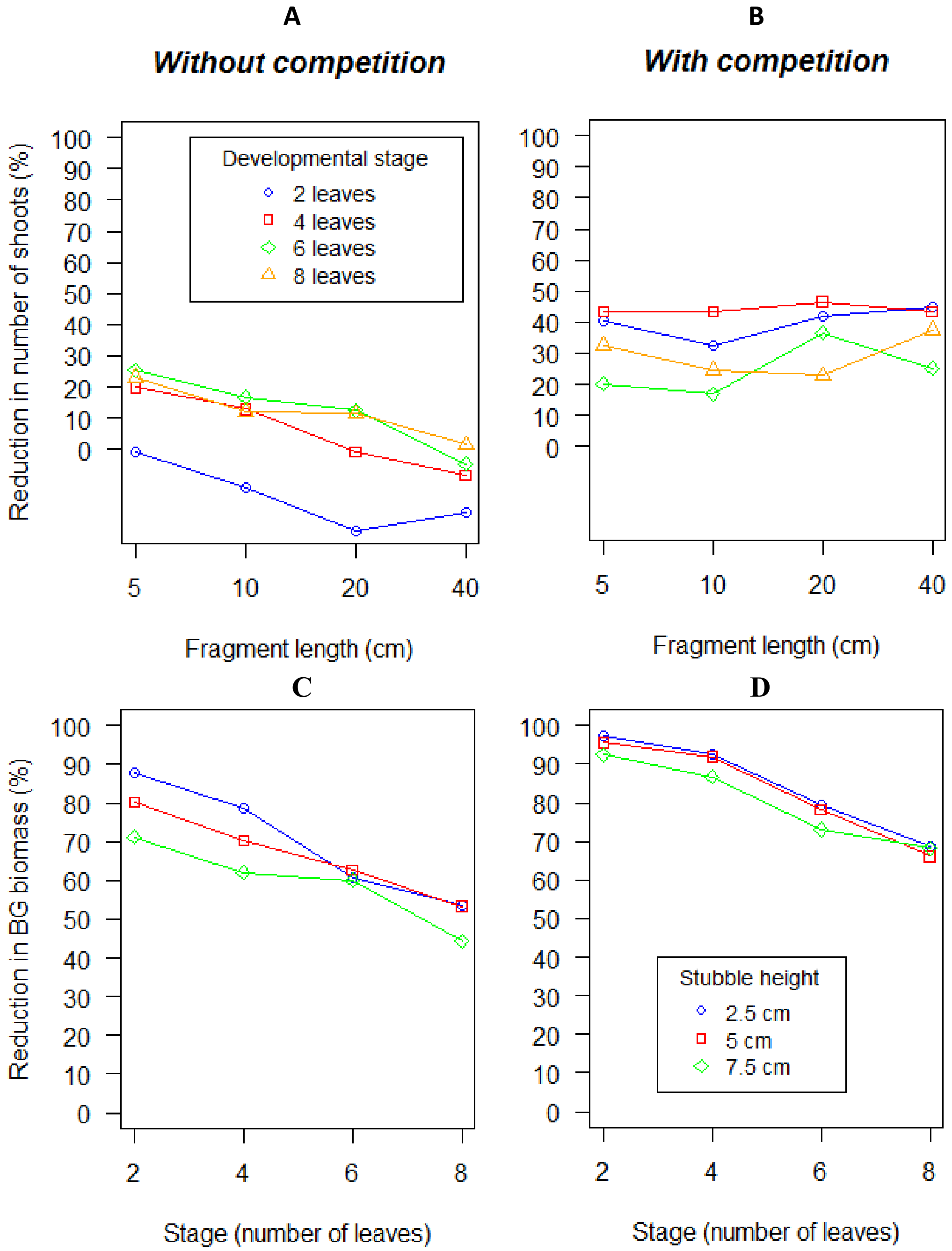

Figure 10: Mean reduction in number of final shoots in *E. repens* by fragmentation to different degree (8x5, 4x10, 2x20, 1x40cm) and cutting at different developmental stages, with (A) and without competition (B) in relation to reference treatments (0%) without cutting (n = 22/24). Reduction in AG and BG biomass in *E. repens* by cutting at different stubble height and different developmental stages with (C) and without competition (D) in relation to reference treatments without cutting (n = 22/24).

Cutting resulted in a greater reduction in number of shoots when competition was present than when it was absent, except one of the treatment combinations (highest degree of fragmentation + cutting at next latest developmental stage) (figure 10). There seemed to be no control effect after lowest degree of fragmentation or when cutting was conducted at 2-leaf stage. For all other treatments this relative comparison points to an increased control of *E. repens*. There was a greater difference between different degrees of fragmentation when competition was absent (figure 10).

Cutting seemed to result in a greater reduction in BG biomass when competition was present than when it was absent, except when cutting was conducted at lowest stubble height and latest stage (figure 10).

4.4.3 Quantitative carbohydrate analyses

Table 6: Degrees of freedom and *P*-values from ANCOVA for terms of the final models fit to concentration (% of DW) of simple sugars and fructan in aboveground (AG) and belowground (BG) plant material, in *E. repens*, (n = 48 = 3(replications) * 16(treatment combinations)), control variables, experimental factors, (-) for non-significant interactions not included in the models, significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.

	Simple sugars				Fructan			
	AG		BG		AG		BG	
	Df	*P*		*P*		*P*		*P*
Replication	2	2e-09 ***	2	0.222	2	0.706	2	2e-05 ***
Fresh weight	1	0.100	1	0.073	1	0.136	1	0.336
Fragmentation	1	0.530	1	0.718	1	0.421	1	0.749
Stage when cut	1	9e-07 ***	1	0.400	1	2e-12 ***	1	1e-10 ***
Stubble height	1	0.003 **	1	0.257	1	0.029 *	1	0.037 *
Competition	1	0.006 **	1	0.054	1	2e-07 ***	1	3e-09 ***
Stage:comp	1	0.007 **	-	-	-	-	1	0.002 **
Fragment:stage	-	-	-	-	1	0.008 **	-	-
Stage:stubble	-	-	-	-	-	-	1	0.012 *
Fragment:comp	-	-	-	-	-	-	1	0.015 *
Fragment:stage:comp	-	-	-	-	-	-	2	0.021 *

Simple sugars in aboveground plant material

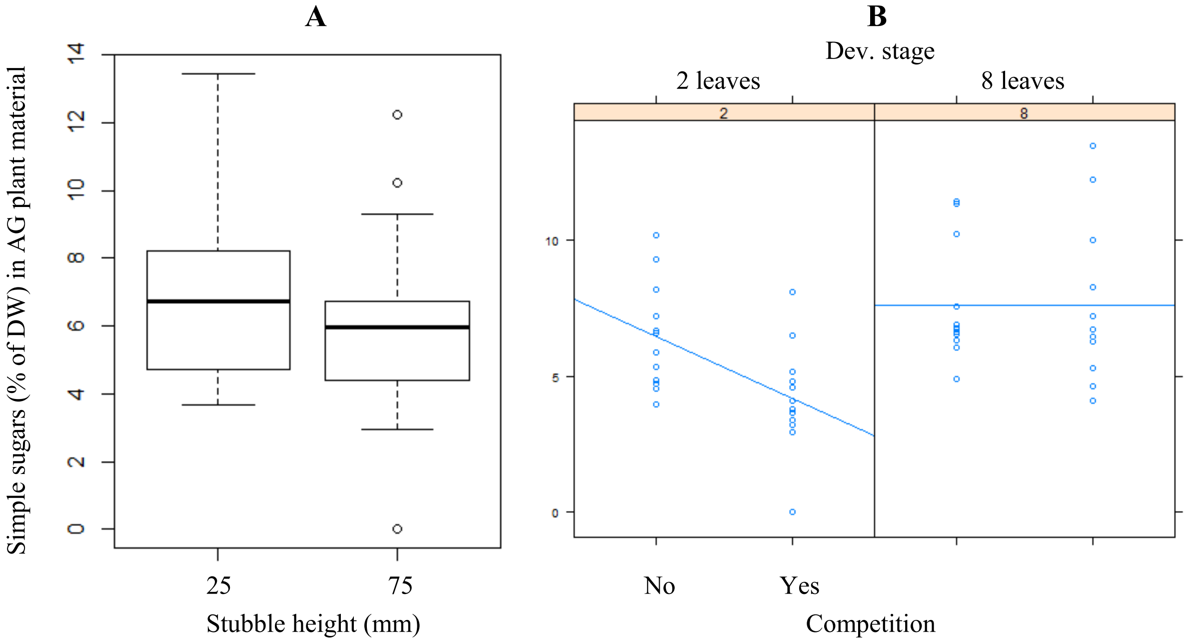

Figure 11: Concentration of simple sugars in AG plant material after cutting at low and high stubble height (A). Interaction between developmental stage when cut (2- or 8-leaf stage) and competition (yes or no) in concentration of simple sugars in AG plant material in *E. repens* (B), (n=24).

Cutting at high stubble height gave lower concentrations of simple sugars in AG plant material (figure 11A). The same did cutting at early developmental stage (figure 11B). After cutting at 2-leaf stage, concentrations of simple sugars were lower when competition was present, while it was not influenced by competition after cutting at 8-leaf stage, as shown by the significant interaction between competition and developmental stage when cut (table 6, figure 11B).

The visible deviation from the mean in the interaction plots is due to treatment effects (16 different combinations) in addition to experimental error and control variables (replications, fresh weight).

Simple sugars in belowground plant material

None of the explanatory variables had any significant effect on concentration of simple sugars (% of DW) in belowground plant material.

Fructan in aboveground plant material

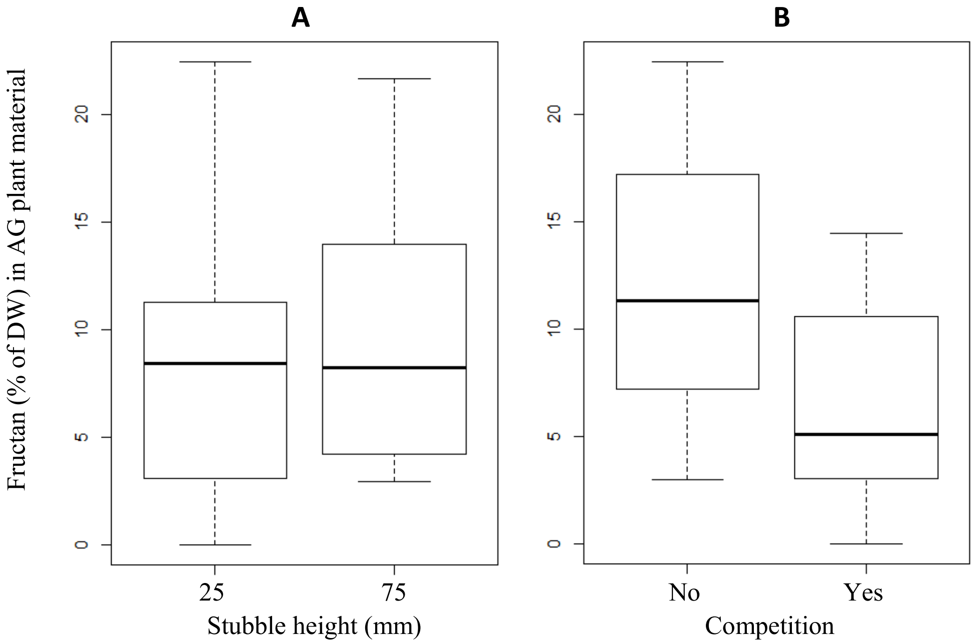

Figure 12: Fructan concentration in AG plant material of *E. repens* following cutting at low (25 mm) or high (75 mm) stubble height (A) and conditions with or without competition (B) (n=24).

Fructan concentrations in aboveground plant material were higher after cutting at low stubble height than after cutting at high stubble height (figure 12A). Competition by clover (figure 12B) and cutting at early developmental stage (figure 13) resulted in lower fructan concentration. At early developmental stage (= high cutting frequency), fragmentation of the rhizomes gave higher fructan concentrations (% of DW), while at late developmental stage (=low cutting frequency) fragmentation

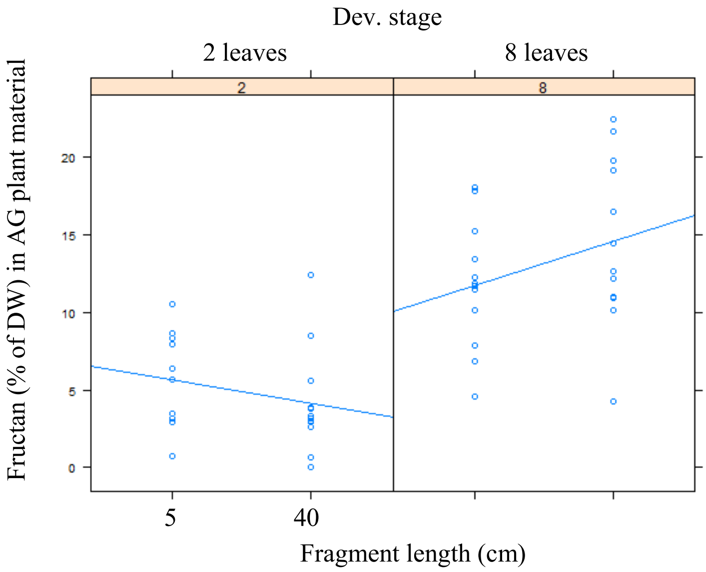

Figure 13: Interaction between degree of fragmentation (8x5 cm/ 1x40 cm) and developmental stage when cut (2 leaves/ 8 leaves) in fructan concentration in aboveground plant material of *E. repens* (n=24).

gave lower fructan concentrations in AG plant material, as shown by the significant interaction between fragmentation and developmental stage when cut (table 6, figure 13). Cutting at early stage gave lower fructan concentrations in AG plant material in general (table 6, figure 13).

Fructan in belowground plant material

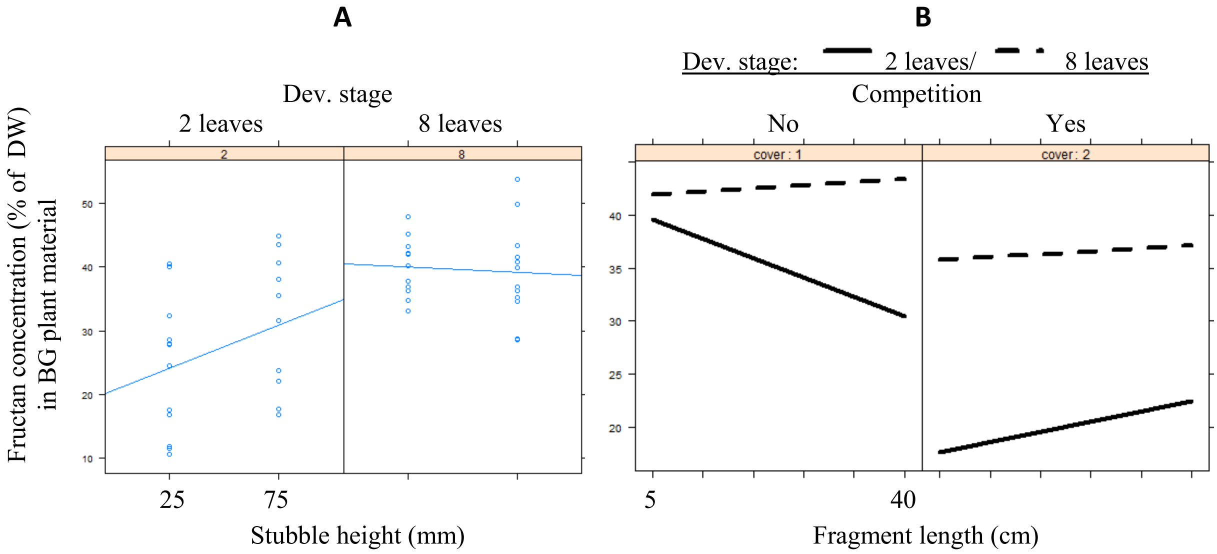

Figure 14: Interaction between stubble height (25/ 75 mm) and developmental stage when cut (2 leaves/ 8 leaves) in fructan concentration in BG plant material of *E. repens* (A); Interaction between competition (no/ yes), fragment length (8x5 cm/ 1x40 cm) and developmental stage when cut (2 leaves / 8 leaves) in fructan concentration in BG plant material of *E. repens* (B) (n=24).

When cut at early stage fructan concentration in belowground plant material was lower at low stubble height, while cutting at late developmental stage gave an unchanged high or even slightly higher fructan concentration at low stubble height than at high stubble height, as shown by the significant interaction between stubble height and developmental stage when cut (table 6, figure 14A). Earlier developmental stage gave lower fructan concentration across stubble height (figure 14A). When competition was present, fragmentation reduced fructan concentration for both stubble heights, while when competition was absent, fragmentation reduced fructan concentration just slightly for late stage (low cutting frequency) and even increased it for early stage (high cutting frequency), as shown by the interaction between fragmentation, competition and developmental stage when cut (table 6, figure 14B). When the rhizomes were highly fragmented, fructan concentration was much lower for cutting at early stage than for cutting at late stage when competition was present, compared to when competition was absent. When competition was present fructan concentration was much lower when cut at early stage than when cut at late stage regardless of fragmentation (figure 14B). Fructan concentration was reduced by fragmentation when competition was present, while it increased (early stage) or showed a negligible reduction (late stage) without competition (figure 14B). When competition was present, fructan concentration was lower within the same level of developmental stage when cut regardless fragmentation or not (figure 14B).

Reduction in carbohydrate concentration after cutting vs no cutting

Fructan concentration was more affected by cutting treatments relative to references than concentration of simple sugars, and concentration of carbohydrates in AG plant material was more affected by cutting treatments than in BG plant material (figure 15). When cutting was conducted at 8-leaf stage BG fructan concentration seemed to increase, while all other cutting treatments seemed to result in reduction of carbohydrates in *E. repens*. Note that there were no significant treatment effects in simple sugars in BG plant material (table 6, figure 15B).

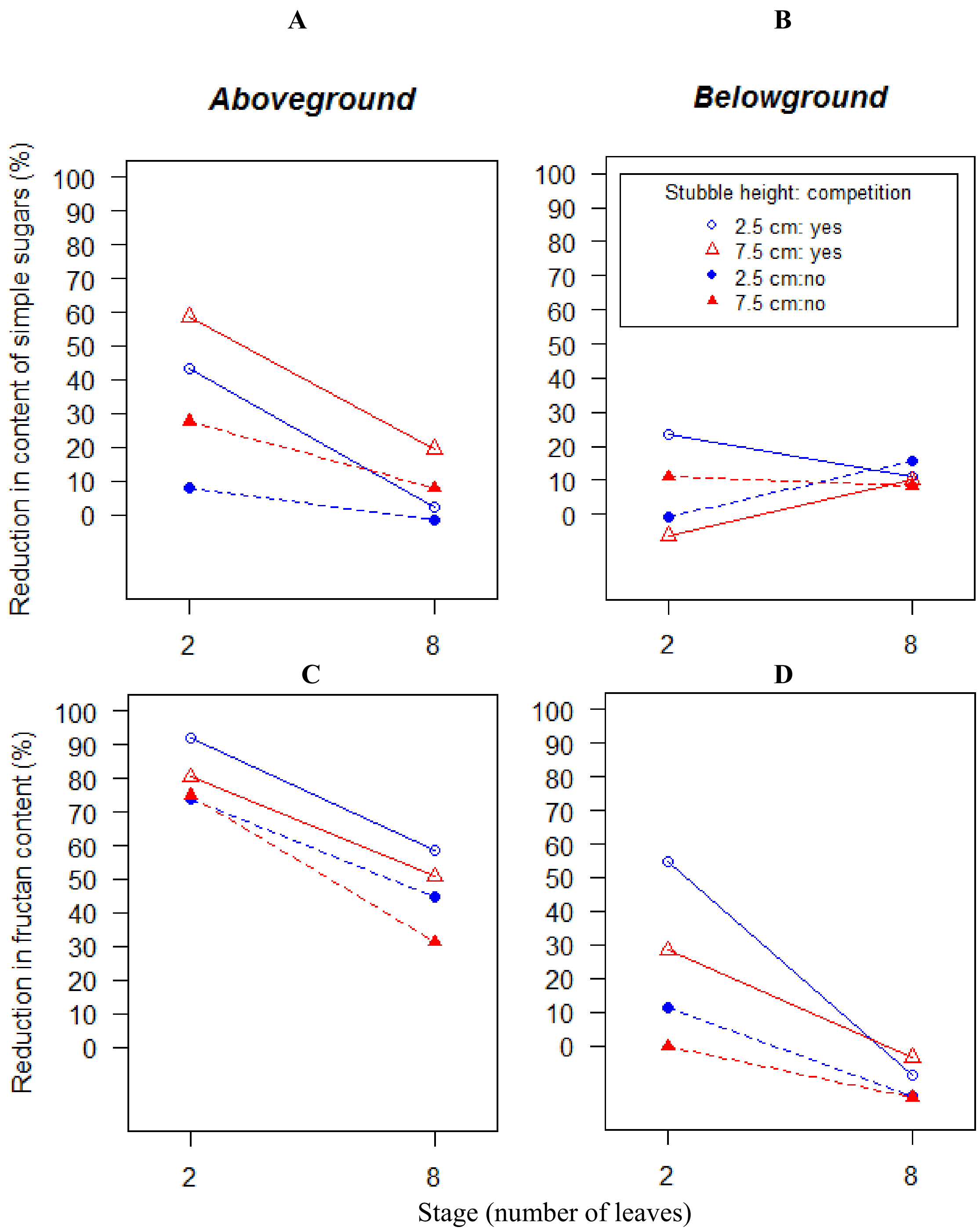

Figure 15: Average reduction in concentration of simple sugars (A and B) and fructan (C and D) (% of DW) in AG (A and C) and BG (B and D) plant material of *E. repens* by cutting and competition compared to reference treatments without cutting (0%), (n= 6)

4.4.4 Experimental and analytical aspects

Judgment – arbitrary assessment of developmental stage

The arbitrary assessment of number of shoots was closest to the real number of shoots of main shoots without competition for 4-leaf stage, of largest shoot per pot without competition for 6-leaf stage and of largest shoots per pot without competition in replication with highest developmental stage for 8-leaf stage (table 7).

Table 7: Mean real number of leaves per shoot in *E. repens*, compared to arbitrary assessment prior to cutting at the predefined stages (n), variation in n due to missing values (failed growth)

Average number of leaves per shoot	4-leaf stage	6-leaf stage	8-leaf stage
All shoots	3.25(139)	3.40(67)	3.61(70)
Main shoots	3.54(139)	4.22(67)	4.72(70)
Largest shoot per pot	4.21(139)	5.55(67)	6.43(70)
Largest shoot per pot, replication 1	4.59(46)	6.00(23)	7.00(24)
All shoots without competition	3.22(72)	3.12(34)	3.27(35)
Main shoots without competition	3.63(72)	4.18(34)	4.60(35)
Largest shoot p. pot without competition	4.50(72)	5.97(34)	7.17(35)
Largest p. pot without competition, rep. 1	4.75(24)	6.64(11)	7.83(12)

Qualitative carbohydrate analysis

There were simple sugars (sucrose, fructose) and fructan present in the plant material (figure 16). Glucose was not visible with this method, but expected to be present. As the columns in the middle of the plate were quite weak, most fructan was high DP fructan (intense column in the bottom) with a distinct separation from simple sugars (intense columns on top).

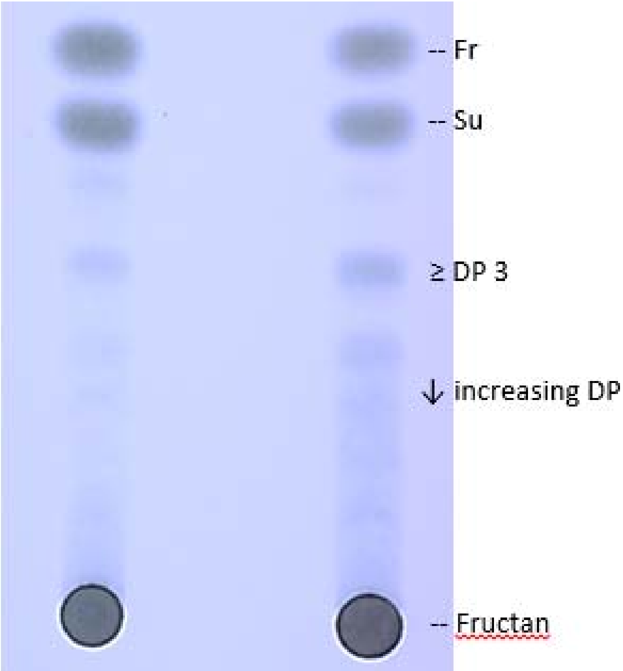

Figure 16: Thin-layer chromatographic separations of water-soluble carbohydrates (fructose (Fr), sucrose (Su) and) fructan in aboveground (left) and belowground (right) plant material of *E. repens*.

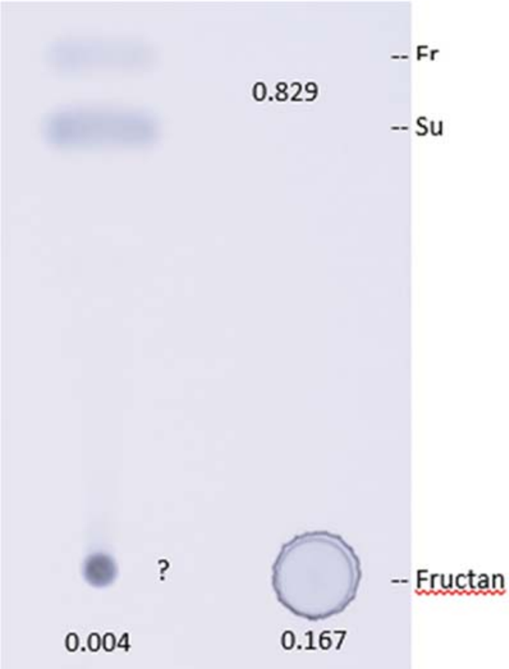

Figure 17: Thin-layer chromatographic separations of ethanol extract (left) and water extract (right) from aboveground plant material of E. repens with relative concentration of simple sugars (fructose (Fr), sucrose (Su) and glucose), fructan and unknown substance (?).

Accuracy of extraction method

The relative concentration of unknown substance in the extracts was very small (figure 17). If the unknown substance would have been fructan, 2.18 % of the total fructan would have remained undetected.

Judgment of use of pooled belowground plant material

There was a significantly higher concentration of simple sugars in new rhizomes compared to initially buried rhizomes (figure 18). Fructan concentration of belowground plant material was significantly higher after mild treatment than after tough treatment regardless of the rhizome fraction.

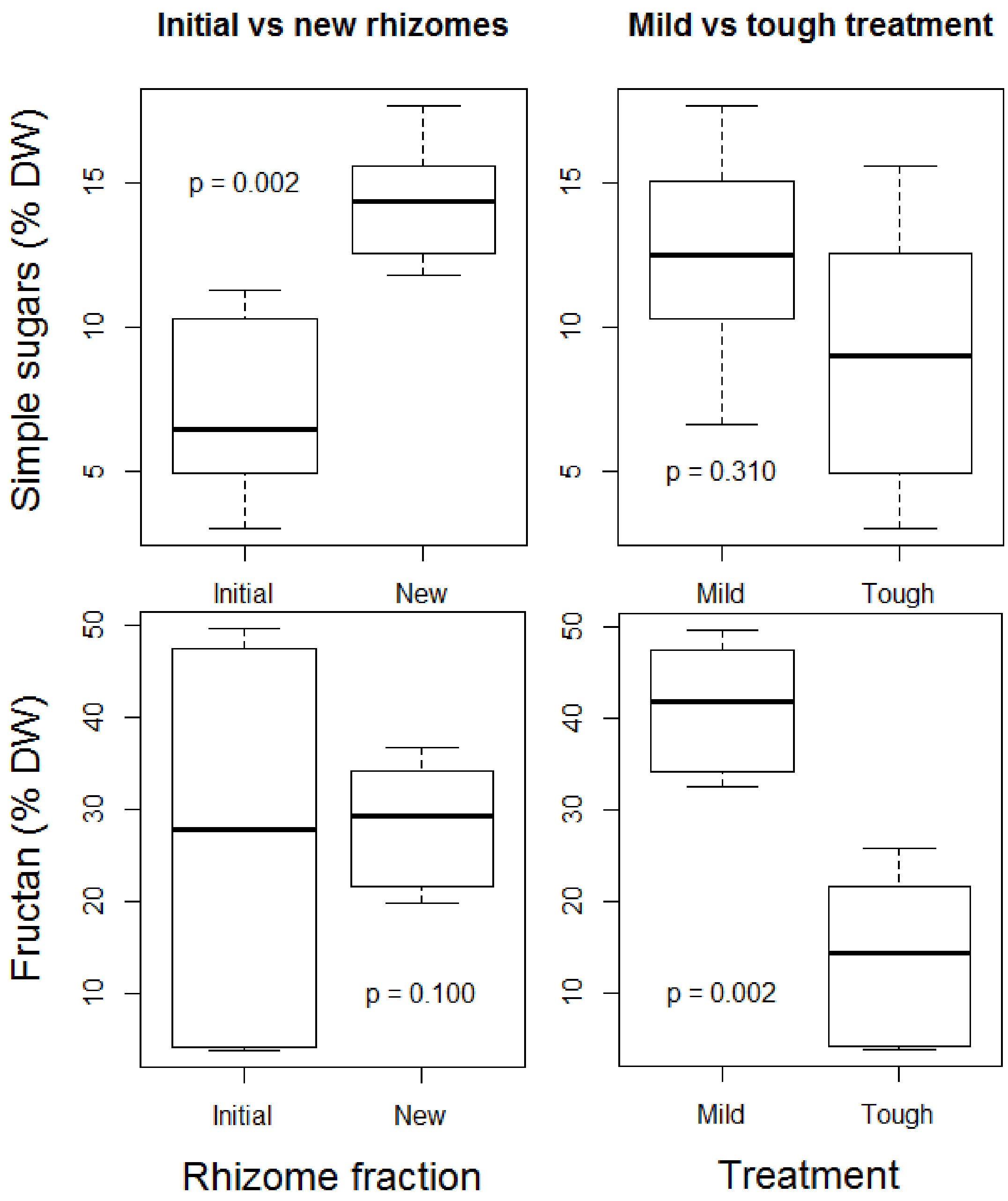

Figure 18: Concentration of fructan and simple sugars (% of DW) in initial compared to new rhizomes of *E. repens* after mild compared to tough treatment with *P*-values from Wilcoxon rank sum test (n = 12).

4.5 Discussion

4.5.1 Fragmentation

Fragmentation influenced number of primary main shoots (table 4, figure 6A), number of final shoots (table 5, figure 7A) and aboveground biomass (table 5, figure 8A), but there was no clear linear relationship between degree of fragmentation and those responses. This is in contrast to Permin (1973) who found a more strict relationship between fragmentation and number of aerial shoots and Turner (1968) who reported less production of new rhizomes and more severe depletion in reducing sugars (glucose, fructose) with higher grade of fragmentation. Regrowth capacity in rhizome fragments buried by ploughing has been reduced with increasing grade of fragmentation (Turner, 1968; Permin, 1973; Håkansson, 1974; Fykse, 1983).

To explore the effect of fragmentation early shoot growth might be preferred instead of later recordings, because *E. repens* continues formation of new shoots throughout the season (Brandsæter et al., 2012) and later development might be influenced by other factors. Håkansson (1974) described the formation of primary shoots in spring to last for approximately 3 weeks. In this experiment one could expect a faster development, because it was established later in the season. The number of shoots was recorded at 4-leaf stage 24-29 days after establishment in July 2012 and 19-21 days after establishment in June 2013. The number of shoots recorded at that time included actually some secondary shoots (tillers). One reason for missing linear relationship between fragmentation and number of primary shoots (figure 6A) might have been that the rhizomes were exposed to light during lasting establishment of the experiment (see 4.5.5). This might have broken apical dominance and induced formation of a great number of shoots independently from fragmentation.

4.5.2 Competition

Competition had a reducing effect on number of primary shoots (table 4, figure 6B and C), number of final shoots (table 5, figure 7B), AG biomass (table 5, figure 8B) and BG biomass (table 5, figure 9B). Reduction in number of primary main shoots by competition might have been a consequence of allelopathy, growth inhibiting effects on other plant species. In white clover there has been found allelopathy in growing plants and herbage of white clover (Macfarlane et al., 1982; Carlsen et al., 2012). The results in this experiment suggest the presence of allelopathic compounds already during early growth or even germination.

Brandsæter et al. (2012) did not find any significant effect of undersown red clover (*Trifolium pratense* L.) on number of shoots in *E. repens*. In these trials the competition by clover was compared to growth of *E. repens* in cereals alone, which might have had a competing effect itself. Swedish experiments with red fescue undersown in winter wheat reduced rhizome biomass in *E. repens* by 40 % without any significant reduction in wheat yield (Bergkvist et al., 2010). The latter results supported Dyke & Barnard (1976) who obtained 29-88 % reduction in pooled biomass of *E. repens* with broad red clover and 43-62 % reduction with ryegrass undersown in barley. Goul Thomsen et al. (2011) found competition from green manure to be the most important factor in experiments combining different root length, burial depth, cutting and competition in control of *Cirsium arvense*, regarding number of shoots, AG and BG biomass. Competition has been found to weaken or even eradicate *E. repens*' ability to form new rhizomes, due to its sensitivity to low light levels (Håkansson, 1974).

Competition also had a reducing effect on concentrations of simple sugars in AG plant material (table 6, figure 11B), and on fructan concentrations in AG (table 6, figure 12B) and BG plant material (table 6, figure 14B). If competition is connected to light, less photosynthesis along with continuing growth in *E. repens* would lead to less accumulation of fructan in rhizomes. Storage carbohydrates in general and fructan in grasses accumulate when environmental conditions are optimal for photosynthesis, but not for growth (Youngner, 1972; Pollock, 1984; Kinmonth-Schultz & Kim, 2011). In this case carbohydrate supply exceeds the demand for growth, allowing accumulation of storage. An exception is reserve storage competing with growth in autumn. Chatterton et al. (1986) found that low temperature favored fructan allocation for storage in *Agropyron ssp* and that this effect was more pronounced with shorter photoperiod. Solhaug (1991) got the same results for *Poa pratensis*. In *E. repens* sprouting capacity and belowground biomass is increasing when photoperiod is decreasing during autumn (Boström et al., 2013). As sprouting capacity has been associated with concentration of storage reserves in *E. repen*s rhizomes (Håkansson, 1967; Leakey et al., 1977) and photosynthesis is decreasing in autumn, the storage of fructan during autumn might compete with growth (Chapin et al., 1990). Accumulation of fructan is then not classified as accumulation of access photoassimilates, but must be classified as reserve storage. This agrees with observations of no development of new rhizomes *E. repens* in autumn. At that time of the year growth is restricted to old rhizomes (Håkansson, 1974). Competition seemed to be more important in control of BG biomass than of AG biomass (figure 10), in line with Håkansson (1974).

4.5.3 Cutting

Interpretation of AG biomass results in this study were impaired by differences in regrowth time span, depending on developmental stage when cut. Therefore stubble height and developmental stage where defined as control variables and no attention was paid to their significances in relation to AG biomass.

Cutting and regrowth capacity

Relative to references, cutting treatments influenced fructan concentration more affected than concentration of simple sugars, and concentration of carbohydrates was more affected by cutting treatments in AG plant material than in BG plant material (figure 15). This was in line with Halling (1988) who found that concentrations of simple sugars in timothy were not influenced by treatment, storage organ or time of the season, while fructan concentrations varied.

Fructan concentration might be used as a measure of regrowth capacity (Chapin et al., 1990). Morvan-Bertrand et al. (1999) reported a strong relationship between early regrowth and fructan concentration in the stubble at the time of defoliation in *Lolium perenne*. And Halling (1988) found a significant positive relationship between autumn fructan concentration and relative growth rate in the subsequent spring in shoots and stem bases of timothy.

Stubble height

Decreasing stubble height had a decreasing effect on BG biomass (table 5, figure 9A), but showed no influence on number of shoots (table 5). This is in line with Youngner (1972) who stated that a lower stubble height gave a lower weight of rhizomes in grasses in general. Also Harrison & Hodgson (1939) found that decreasing stubble height resulted in decrease in weight of rhizomes, roots and aboveground biomass during a 7 weeks period with weekly defoliation treatment, even though *E. repens* was amongst the grasses with a better ability to adapt to severe defoliation and continued production of rhizomes after cutting treatment, probably at the expense of root production.

Low stubble height led to higher concentrations of simple sugars (table 6, figure 11A) and fructan (table 6, figure 12A) in AG plant material, but to lower concentration of fructan in BG plant material (table 6, figure 14A), when cutting was conducted more frequently. The higher carbohydrate concentrations (AG) at low stubble height than high stubble height might be a result of the choice of response. A reduction in carbohydrate content with low stubble height was conceivable, if carbohydrate content was calculated as pool size. May (1960) reviewed reserve carbohydrates to be the foundation of post-defoliation respiration in belowground plant parts and early aboveground regrowth. Brown (1943, cited in Youngner, 1972) reported decrease in rhizome weight with decreasing stubble height during summer following biweekly defoliation in *Poa pratensis*, but the opposite in the subsequent autumn. The reason for that might have been the use of rhizome reserves for production of new aerial shoots during summer, leading to the decrease in rhizome weight; followed by higher level of photosynthesis of a greater herbage pool and reallocation of reserves to rhizomes in autumn. In addition this seems to be in line with the general seasonal fluctuations in carbon concentration in cold-season species in autumn when temperature is lower and days are shorter. Chatterton et al. (1986) found that low temperature favored fructan allocation for storage in *Agropyron ssp* and that this effect was more pronounced with shorter photoperiod. Solhaug (1991) got the same results for *Poa pratensis*. In *E. repens* sprouting capacity and belowground biomass is increasing when photoperiod is decreasing during autumn (Boström et al., 2013). As sprouting capacity has been associated with concentration of storage reserves in *E. repen*s rhizomes (Håkansson, 1967; Leakey et al., 1977) and photosynthesis is decreasing in autumn, the storage of fructan during autumn might compete with growth. Accumulation of fructan is then not just an accumulation of access photoassimilates, but must be regarded as reserve storage according to Chapin et al. (1990).

Cutting frequency – developmental stage when cut

Increasing cutting frequency reduced BG biomass (table 5, figure 9B) in the subsequent autumn. This is in line with Youngner (1972) who stated that a higher frequency gave a lower weight of rhizomes in grasses in general. Also Dexter (1936) and Håkansson (1974) described that mowing has been shown to be most effective with short interval. High cutting frequency alone seems to stimulate formation of more, but weaker shoots. Number of shoots was increasing with increasing cutting frequency (table 5, figure 7B). If high cutting frequency is combined with competition, number of shoots is not increasing and biomass is reduced (figure 7B) in a synergistic way. Brandsæter et al. (2012) found a significant decreasing effect of mowing on number of shoots in *E. repens* in one of two experimental seasons. In their study, however, the cutting was only conducted one single time, in addition to the cutting effect of the combine harvester. Holmøy & Teslo (2000) found that two postharvest cuttings were more effective against weeds than one single cutting, even though not as effective as stubble tillage. With increasing frequency of defoliation, Turner (1968) obtained decreasing growth and biomass of new rhizomes in *E. repens*. With biweekly defoliation (4 harvests) new rhizome growth was depressed totally.

High cutting frequency reduced concentration of simple sugars in AG plant material (table 6, figure 11B) and concentration of fructan in AG (table 6, figure 13) and BG plant material (table 6, figure 14A) in the subsequent autumn. Increasing fructan concentration after 1 cutting treatment at 8-leaf stage, compared to reference treatments without cutting (figure 15B and D), was in line with results by Hogg & Lieffers (1991) who found that belowground carbohydrate reserves in rhizomes of *Calamagrostis canadensis* plants mowed once were significantly higher than of unmown plants, while they were significantly lower in rhizomes of plants mowed several times during the season. Henskens (1993) found the same pattern for *E. repens*. After a single defoliation, regrowth was found to be supported by photoassimilates originating from remaining herbage, while after repeated defoliation storage reserves from rhizomes were relocated to regrowth. As 8-leaf stage cutting in this study was conducted late and as the only cutting, this might be in line with less effective weed control observed when mowing was conducted only once, in addition to combine harvesting (Brandsæter et al., 2012). In general fructan allocation takes place when growth conditions are limited, but photosynthetical supply of assimilates is present. After defoliation regrowth is competing with fructan reserve storage and suggested to reduce fructan concentration (Pollock, 1984). Gräβler & Von Borstel (2005) described fluctuations in fructan concentration in aboveground biomass of a number of pasture grasses. During a growing season with 5 harvests fructan concentration was initially increasing, reaching a minimum at 3rd harvest and increasing above initial values at the last harvest on average for all examined grasses. *Elymus repens* as an exception did not show a distinct decline in fructan concentration after 3rd harvest in midseason.

4.5.4 Interactions - combined control strategies

Fragmentation and other treatments

Although fragmentation, as a main factor, did not influence any carbohydrate concentration (table 6), it was part of interactions with other factors, having main effects, in fructan concentration in AG and BG plant material (table 6, figures 13 and 14B). High degree of fragmentation seemed to increase fructan concentration compared to low level of fragmentation, when combined with high cutting frequency without competition. This might be interpreted as a kind of survival strategy: under tough conditions more fructan is allocated to approximately the same (AG) or less (BG) biomass (table 5, figures 8A, 9B) or as a consequence of expressing regrowth capacity as fructan concentration (% of DW). A reduction in fructan content with high degree of fragmentation was conceivable, if fructan content was presented as pool size instead. When there was competition in addition to the high cutting frequency, fructan concentration in BG plant material was at its lowest with fragmentation and generally lower than without competition (table 6, figure 14B). That would mean that the survival strategy was not good enough when *E. repens* was exposed to an additional control measure. This might be exploited in a starvation strategy with combined control measures. The rhizomes` exposure to light during trial establishment (see 4.5.5), might have made it impossible to detect an interaction between fragmentation and cutting (table 5) in BG biomass. According to the results of Permin (1960; 1973), Håkansson (1974) and Brandsæter et al. (2012) an increasing reduction in AG and BG biomass was expected with increasing fragmentation combined with increasing cutting frequency (decreasing developmental stage). The higher the grade of fragmentation, the higher the amount of aerial shoots and the weaker each of them. If the fragmentation is combined with mowing of the aboveground plant parts before or at compensation point, photosynthesis is limited and so is the available energy for the induced shoots. The storage reserves in the rhizome fragments are depleted and regrowth capacity of the fragments is weakened, which means that one would have expected less BG biomass and less fructan in the plant material. Another explanation for the missing interaction between fragmentation and cutting might have been the fact that there were no non-fragmented treatments included. The lowest degree of fragmentation in reality was a fragmentation, too, because the rhizomes were cut from a bigger system of rhizomes in the field. In fact it is impossible to include a non-fragmented reference in a pot experiment of this type. There might theoretically be a bigger difference between no fragmentation and fragmentation in general than between different degrees of fragmentation. There is a need to further investigate the effect of rhizome fragmentation in an undisturbed system. There seemed to be a greater difference in attained control, in number of final shoots, between different degrees of fragmentation relative to reference treatment without cutting, when competition was absent (figure 10A and B). Those rhizome fragments which were not fragmented additionally, gave an increase in number of shoots, relative to reference without cutting, when competition was absent, as did several of the other combinations of fragmentation and developmental stage when cut (figure 10A). The highest degree of fragmentation was the only one which did not lead to increased number of shoots combined with cutting at any of the developmental stages when competition was absent (figure 10A). This is in line with the mentioned literature, as the lowest degree of fragmentation, combined with cutting and competition, did not deplete the rhizomes as much as the higher ones did and more of the induced aerial shoots were able to reach the soil surface and survive.

Competition and cutting frequency (developmental stage)

In this experiment there was an interaction between competition and developmental stage when cut in number of final shoots (table 5, figure 7B) and BG biomass (table 5, figure 9B), as well as in concentration of simple sugars in AG plant material (table 6, figure 11B) and of fructan in BG plant material (table 6, figure 14B). When competition was absent, a higher cutting frequency stimulated formation of aerial shoots (table 5, figure 7B) and increased concentration of simple sugars in AG plant material (table 6, figure 11B) and fructan in BG plant material (table 6, figure 14B), while BG biomass decreased (table 5, figure 9B). This might be interpreted as a kind of survival strategy: under really tough conditions more, but weaker shoots are produced and more carbohydrates are allocated to less biomass. On the other hand a reduction in fructan content with high cutting frequency was conceivable, if fructan content was presented as pool size, instead of as concentration (% of DW). When competition was present, those responses were almost not affected or slightly reduced by high cutting frequency, but generally lower. Here again the survival strategy was not good enough when *E. repens* was exposed to an additional control measure. This interaction is similar to the interaction between fragmentation and other treatments which has been described in connection to the starvation method: induction of more but weaker aerial shoots is combined with cutting and/ or competition resulting in a more effective control of *E. repens* (Permin, 1960; 1973; Håkansson, 1974; Brandsæter et al., 2012).

Stubble height and cutting frequency (developmental stage)

Low stubble height led to lower concentration of fructan in BG plant material, when cutting was conducted more frequently, while it almost did not affect the concentration, when cutting was conducted only once (table 6, figure 14A). This is not really in line with Youngner (1972) who described a general complementary relationship between stubble height and mowing frequency.

Competition and cutting in general

Compared to references, competition seemed to act together with cutting in a depleting strategy following induction of more aerial shoots by fragmentation of the rhizomes (figure 10A and B) and competition led to a greater reduction in AG and BG biomass for almost all combinations of stubble height and developmental stage when cut, than treatment without competition (figure 10C and D). In comparison with reference treatments without cutting treatment, cutting seemed to be more important than the stubble height or the developmental stage at cutting for number of shoots and biomass (figure 10). Brandsæter et al. (2012) did not recognize any interaction between mowing and competition. That was in contrast to Goul Thomsen et al. (2011) and Graglia et al. (2006) who found greater control effect of cutting in *Cirsium arvense* when the weed was exposed to competition. Although mowing has been assumed to be a weak control measure compared to soil tillage, it might be used as an important part of a combined control strategy (Brandsæter et al., 2012; Goul Thomsen et al., 2014), especially under reduced tillage conditions where the stronger soil tillage control methods are not in use. It might have been advantageous to design the experiment with several degrees of competition included.

4.5.5 Experimental and analytical aspects

Judgment – arbitrary assessment of developmental stage

The arbitrary assessments and the cutting treatments were conducted for all plants at the same time, even though there were differences in plant development between those with competition and those without, and between replications. According to the comparison (table 7), the involved staff had poor ability to judge the shoots' developmental stage. This shouldn`t have had any influence on the reliability of the results and it is still possible to discuss the results in view of increasing developmental stage instead of specific stages. One could believe the discrepancy was caused by emphasis on the main shoots or the largest shoots in each pot, but that did not explain the deviance. Most importance might have been attached to the shoots without competition (which were easier to discover) or the shoots in the first replication (which was the 1st one established with hence highest developmental stage), but that did not give the complete explanation either. The reason for the deviance seemed to be the combination of these challenges. Assessments seemed to become more imprecise with increasing developmental stage. Variation between shoots is increasing throughout the growing season, due to steady development of new shoots in *E. repens* (Brandsæter et al., 2012). In addition to the author, the assessments were discussed and implemented by several experienced persons. One should not expect that the involved persons` ability to judge the developmental stage arbitrarily was that poor. These results might show that the great variation in developmental stage of *E. repens* shoots, due to tillers and secondary shoots, complicate decisions for onset of experimental and real life measures to a large extend.

Qualitative carbohydrate analyses

The fructan showed a DP of more than 12 (figure 16), which is in line with Arni & Percival (1951) and Hammer & Morgenlie (1990) who reported heavily branched structure for fructan in rhizomes of *E. repens* and suggested a DP of 30 (Arni & Percival, 1951). The distinct separation of simple sugars and high DP fructan on the TLC plate excludes underestimation of the fructan portion after extraction with ethanol (Smouter & Simpson, 1989). A standard species could have been included in the TLC, e.g. *Helianthus tuberosus*, for more accurate comparison of bands of different DP.

Judgment - accuracy of the extraction method

Simple sugars were nicely separated from fructan, but some unknown substance remained in the fructan region (figure 17). Quantitative analysis of this remaining substance showed that it had little impact on the fructan concentration results. If the unknown substance was fructan 2.18 % of the total fructan would remain undetected and the impact on the result would have been negligible. The unknown substance might have been protein or starch. In addition to fructan there have been found small amounts of starch in cold season grasses (Smith, 1973 and references herein). Arni &

Percival (1951) and Hammer & Morgenlie (1990) used chloroform and butanol to remove proteins from *E. repens* water extract prior to fructan analysis.

Judgment – use of pooled belowground plant material

When the experiment was concluded it was not feasible to relocate all initial rhizomes in all pots, especially in the pots with high degree of fragmentation. To use pooled belowground plant material in the quantitative carbohydrate analysis could have led to methodological error and misleading results if there was an existing difference in carbohydrate concentration between initial and new rhizomes. Additional analysis showed that the type of treatment had a greater impact on fructan concentration of the plant tissue than the rhizome fraction (figure 18). There was no difference in fructan concentration between initial and new rhizomes. On the other hand, the concentration of simple sugars was influenced mostly by whether the rhizomes were old or new. There was no difference in concentration of simple sugars between mild and tough treatment. As it seems to be the fructan concentration which is important for regrowth capacity in *E. repens* the used method probably had no impact on the results reliability.

Duration of establishment

Even though the three replications in 2013 were established at successive days, in 2012 the last replication 3 days delayed, there were significant differences in number of primary shoots, number of final shoots, final AG and BG biomass, concentration of simple sugars in AG plant material and fructan concentration in BG plant material between the replications.

This was taken into account in the analyses, but tells something about *E. repens*` great growth rate and regrowth ability, which should have an impact on experimental practices in experiments with *E. repens* in general.

The duration of establishment might also have had an influence on apical dominance and sprouting. Excavated rhizomes were stored in plastic at 4 °C. During the following days the rhizomes were cut by scissors into 40, 20, 10 and 5 cm pieces and still stored cold until burial. Time consuming preparation and burial of the rhizomes led to lasting exposure to light during the day, repeated several days. Light might have had an impact on physiological processes in the rhizomes during handling and hence an impact on the result. Johnson & Bucholtz (1961) did not record any difference in bud activity and shoot and root growth between rhizome fragments of *E.*

repens grown in vitro with light or without exposure to light. But all their rhizomes had been exposed to light during establishment of the experiment/ preparation of the material. Experiments in *E. repens* have shown that exposure to light breaks apical dominance (McIntyre, 1971). Nyahoza et al. (1974) broke apical dominance when they exposed rhizome tips of *Poa pratensis* to light. Their experiment was established under low light conditions.

The time consuming procedures led in addition to storing time between preparation and burial of up to 10 days. Some rhizomes were developing new adventitious buds or even small shoots from adventitious buds during that time. Optimal fragmentation time would have been right before burial in order to expose all rhizomes to the same conditions during sprouting.

Duration of termination

Also trial termination was lasting whole days and harvested plant material was placed in the oven a few times during the day or just by the end of the day. One could be worried about the impact on concentration of carbohydrates. Fick & Nolte (1986) concluded that enzymatic degradation of nonstructural carbohydrates had no impact on the results in harvested plant material, even 24 hours after collection. Pontis (1990) states that enzymatic degradation must be avoided, that this can be done by extraction in boiling water or preparation in boiling alcohol prior to extraction, and that oven drying at low temperatures does not inactivate enzymes. Smith (1972) states that linear fructan in forage grasses usually is extracted by cold water. Halling (1988) steamed plant material before drying in order to stop enzymatic activity, but Archbold (1938) pointed out that higher temperature than 50 °C might destroy fructose. Trlica & Cook (1971) treated their plant material with 95% ethanol and kept it air-sealed in order to avoid enzymatic degradation.

The results might also have been influenced by diurnal variations in carbohydrate concentration (Holt & Hilst, 1969; Bowden et al., 1968; Longland & Byrd, 2006). Ideally, the harvesting should have been done at the same time of the day for all treatments. The final conclusion must be that this experiment was oversized and/or understaffed.

4.5.6 Conclusions and recommendations

This study cannot give a clear answer to the question (I) about what happens if the rhizomes are fragmented as desired, because experimental design (excluding real non-fragmentation) and choice of responses (carbohydrate concentration instead of pool size) made interpretation of results difficult. There is a need to further investigate the effect of rhizome fragmentation in an undisturbed system. Therefore (II) the relationship between a potential implements' adjustment (grade of fragmentation) and the other treatment factors included in the study (stubble height, cutting frequency and competition) is difficult to explore as well.

Brandsæter et al. (2012) and Goul Thomsen et al. (2014) concluded that mowing is a weak control measure compared to soil tillage, but valuable as part of a control strategy taking into account environmental aspects. On the other hand Turner (1968) found that biweekly defoliation had a stronger reducing effect on BG biomass and concentration of reducing sugars (glucose + fructose) in *E. repens* than different levels of fragmentation and burial depth. Defoliation alone does not inhibit *E. repens* perceptibly, but is an important part of combined control methods (Håkansson, 1974). In cereal production defoliation can be combined with post-harvest soil tillage (Boström et al., 2013). *Elymus repens'* high tolerance to control measures and its great regeneration ability makes it necessary to combine all possible control methods. Non-chemical weed management should include both direct and preventive, cultural and mechanical measures (Melander et al., 2005). In organic agriculture it is of great importance to include weed control as part of the 'multifunctional crop rotation' (Olesen, 1999). And especially in reduced tillage all the measures conducted in this study might be practiced for optimal control of *E. repens*. (III) In this study the different treatment factors and their levels influenced each other in different interactions, essentially in a synergistic way. Exceptions were discussed as possible survival strategy in *E. repens*, which might be exploited in a starvation strategy, or due to choice of response to express regrowth capacity. Among those a new discovery was that high cutting frequency stimulated formation of new aerial shoots.

The relative importance of the different factors in this study were competition, stage when cut, stubble height and fragmentation, ranking from high to low importance. In practical use the ranking might be different if different levels of competition are considered. Even fragmentation might be more important if its effect is related to an undisturbed system lacking fragmentation totally.

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