



# Effect of cultivar mixture on the competitive ability of barley against weeds

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MSc thesis

## Abstract

### Effect of cultivar mixture on the competitive ability of barley against weeds

Estevan, E. SLU, Department of Crop Production Ecology. Master thesis. Uppsala, Sweden.

While it is known that mixtures of cultivars generally stabilise crop yields and reduce losses caused by diseases, their influence on weeds has not yet been thoroughly investigated. Competitive effects against weeds are dependent on specific plant characteristics, which can vary between cultivars. The aim of this study was to investigate whether mixtures of barley cultivars with different characteristics could suppress weeds better than barley grown in pure stands, and whether the weed suppressive effect differed between the mixtures.

A greenhouse trial was performed with three two-rowed spring barley (*Hordeum vulgare* L. spp. *vulgare*) cultivars grown in pure stands, all possible two-cultivar mixtures and the three-cultivar mixture. The barley cultivars Hydrogen, Henni and Troon were chosen because they differ in the three characteristics allelopathic activity, root length development and shoot length in the first growth stages. Turnip rape (*Brassica rapa* cv. Agat) and perennial ryegrass (*Lolium perenne* cv. Helmer) were chosen as model weed flora.

The results indicate that cultivar mixtures can improve the competitive ability of barley, reducing biomass production by weeds and diminishing barley biomass losses. Contrasting allelopathic activity and shoot development characteristics in the mixture increased the competitive effect. The weed suppressive effect differed between mixtures and was lowest in the mixture with differing root development but low shoot development and high allelopathic activity. Mixtures did not express the sum of characteristics of each individual barley cultivar. In fact, on some occasions the mixture that showed the best competitive ability did not contain the cultivar that demonstrated the best competitive ability when grown in pure stand. The mixtures that included cv. Hydrogen, which has high allelopathic activity, improved the competitive response in terms of barley biomass.

Mixture design is needed to get cultivar mixtures that can control weeds. More research is needed on this aspect to devise a formula that allows us to design correct mixtures, and therefore to use cultivar mixing as a method for controlling weeds.

Key words: cultivar mixture, *Hordeum vulgare* L., weed control, allelopathy, competitive ability, mixture design

## Resumen

Efecto de la mezcla de variedades en la habilidad competitiva de la cebada contra las malas hierbas. Estevan, E. SLU, Department of Crop Production Ecology. Master thesis, . Uppsala, Sweden.

Se sabe que mezclando variedades generalmente se estabiliza la adaptabilidad, la producción y las pérdidas del cultivo debidas a enfermedades, pero la influencia de la mezcla de variedades sobre las malas hierbas apenas ha sido investigada. Los efectos competitivos sobre las malas hierbas varían según las características de las plantas y las características varían según los cultivares. El estudio persigue determinar si la mezcla de variedades de cebada podrían reprimir mejor las malas hierbas que cultivando la cebada en una sola variedad, y si el efecto represivo sobre las malas hierbas difiere entre las mezclas.

Se realizó un experimento de invernadero con tres variedades de cebada de primavera (*Hordeum vulgare* L. spp. *vulgare*) cultivadas en líneas puras, mezclas de dos variedades y mezclas de las tres variedades. Las variedades de cebada (Hydrogen, Henni, Troon) fueron seleccionadas según sus características de tolerancia a las malas hierbas: alta capacidad alelopática, gran desarrollo radical y gran alargamiento del vástago en los primeros estados de desarrollo. Como modelo de flora de malas hierbas fueron elegidas dos especies: *Brassica rapa* cv. Agat y *Lolium perenne* cv. Helmer.

Los resultados indican que la mezcla de variedades puede mejorar la capacidad competitiva en la cebada, reduciendo la biomasa producida por las malas hierbas y disminuyendo las pérdidas en biomasa de la cebada. La competencia se aumenta contrastando las características de actividad alelopática y el desarrollo del vástago en las mezclas. La represión sobre las malas hierbas difería entre las mezclas y fue menor en la mezcla con plantas de gran desarrollo de la raíz, bajo desarrollo del vástago y alta actividad alelopática. Las mezclas no muestran la suma de los efectos de las características de cada variedad de cebada individualmente. De hecho, en algunas ocasiones la variedad con mejor capacidad competitiva en monocultivo no compone la mezcla con mejor capacidad competitiva. Las mezclas que contenían el cultivar Hydrogen de alta actividad alelopática, mejoraron la respuesta competitiva en biomasa de cebada.

Se necesita mayor investigación que permita deducir una fórmula para diseñar correctamente las mezclas y que estas puedan ser utilizadas como método de control de malas hierbas.

Palabras claves: mezcla de variedades, *Hordeum vulgare* L., control de malas hierbas, alelopatía, habilidad competitiva, diseño de la mezcla.

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## Abbreviations

CAP	Common Agrarian Policy
CE	Competitive effect
CR	Competitive response
cv	Cultivar (commercial cultivar)
DAS	Days after sowing
DM	European Commission
EC	European Commission
EU	European Union
EU-25	European Union 25 States
Fig	Figure
g	gram
p.	Probability
SAS	statistical analysis system
SLU	<i>Sveriges lantbruksuniversitet</i> , Swedish University of Agricultural Sciences
spp	subspecies

## **Introduction**

The use of the traditional agrarian techniques, in which the high demands of commercial crop production require the use of large amounts of inputs, not only involves over-exploitation of water and soil resources, but also incurs a high risk of environmental contamination due to losses of inputs not consumed by the crop. The continued use of crop protection chemicals and artificial fertilisers over the years has led to accumulation of such compounds in soil and water. Some of these chemical substances are intended to imitate compounds that exist in nature, while the purpose of others is to eliminate animals or plants detrimental to crop production. The latter could therefore have negative effects on human health when present as residues in agricultural produce. As a result, consumers have begun to demand a different type of produce in which environment protection and nutritional safety are guaranteed. Therefore low-input production has been increasingly used in Europe in the past few years, with conventional agricultural practices (high-input) giving way to cultivation under more sustainable conditions. Sustainability has been defined as the capacity to satisfy the needs of the present generation without diminishing the potential of future generations to satisfy their own needs. Sustainable crop production is therefore characterised by reduced inputs of pesticides and synthetic fertilisers and because of this reduces the risks to consumers and the environment, as well as guaranteeing agricultural diversity.

The most rigorous low-input system is the organic farming system, as defined by EU Directive 2092/91 and by the later modifications EU Directives 2381/94 and 1488/97, where pesticides and synthetic fertilisers are generally not allowed and yield stability and quality have to be ensured by the cropping system itself. The development of organic farming has increased in recent years and nowadays this type of cropping is one of the more dynamic agricultural sectors within the European Union. In 1990 it represented only 3% of the total viable agricultural soil in the EU, but between 1993 and 1998, this sector grew by around 25% annually and from 1998, its annual expansion has been up to 30% in some member states (CAP reform, 2003). The European Commission (EC) continued to work towards new regulations and in December 2005 adopted a new standard in this issue, the objective of which was to improve the understanding of organic farming as much for the consumer as for the farmer. In fact the European Union has totally changed its approach to supporting the agricultural sector since the last reform of the Common Agrarian Policy (CAP) in June 2003, which was aimed at defining the perspective of the agricultural market in the EU for the period 2003-2010. The new CAP offers to European farmers the possibility of producing what the market demands, without losing the right to support, since it decoupled the link between subsidisation and production, in order to render the farmers of the EU more competitive. This new 'unique aid by operation' encompasses respect for the environment, nutritional safety and animal welfare norms and therefore markets such as those for sustainable or organic agriculture are clearly relevant.

Ecologists have demonstrated that increasing species diversity contributes to greater ecosystem productivity and stability, while the benefits of genetic diversity within single-species populations have also been demonstrated (Helland & Holland, 2002). Cultivar mixtures are applicable to many agricultural situations, since there are a number of potential benefits from their use in low input and

organic systems (Finckh *et al.*, 2000). One of the most important criteria for the success of mixtures in practice is their yielding ability. It has been argued that mixtures present greater yield advantages in low-yielding environments than in high yielding environments (Finckh *et al.*, 2000).

The use of cultivar mixtures is an epidemic control strategy for diseases. Host mixtures may restrict the spread of diseases relative to the mean of components, provided that the components differ in their susceptibility (Finckh *et al.*, 2000). There are several research groups working on this subject throughout Europe. In the period 1979-1991, the Danish Advisory Centre conducted many trials with spring barley mixtures regarding disease resistance and yield capacity. The conclusion was that barley mixtures were effective against powdery mildew, leaf blotch and scald, common diseases that were reduced significantly in trials with winter barley (Finckh *et al.*, 2000).

While it is known that cultivar mixtures generally stabilise crop yields and reduce yield losses caused by diseases (Finckh *et al.*, 2000), their influence on weeds has not been investigated to any significant extent. The competitive effects of different crop cultivars against weeds vary depending on botanical characteristics, and therefore some such characteristics can determine the competitive advantage. The aim of the present study was to investigate whether mixtures of barley cultivars with different characteristics could suppress weeds better than barley grown in pure stands, and whether the weed suppressive effect differed between the mixtures.

## Literature review

### *Barley*

Barley (*Hordeum vulgare* L.) has been used by Man as a crop for over 15 000 years, and it is thought that it was one of the first plants domesticated for agriculture. Our Neolithic ancestors knew different seeds and cereals and learned that crushed and mixed with water, they form *papilla*, the precursor of the present barley bread. More than 6 000 years ago along the banks of the Tigris and Euphrates rivers, the Sumerians produced and consumed barley beer. Different uses have been found for this cereal during human history and its domestication over centuries means that nowadays, we have at our disposal a large diversity of different cultivars. Thank to this diversity, the most appropriate barley cultivar can be used for each purpose, *e.g.* malting malt to produce beer, high protein barley to get flour to make bread and feed barley for livestock.

Barley is the fourth most commonly grown cereal in the world, after wheat, maize and rice. Its culture is very extended because it is characterised by wide ecological adaptation thanks to its climate requirements being very few, although it grows better in cool and moderately dry climates. Therefore it grows at high altitude and latitude. Of the cereals, barley is the one that adapts best to highest latitudes (when the most precocious cultivars, spring cultivars, are used) and thus this crop has a great adaptation in Sweden. In fact, spring barley is the second most abundant cereal in Sweden after wheat, and the third most abundant crop overall after wheat and sugar beet. In 2005, barley production in Sweden amounted to 1 592 900 tonnes, compared with 2 246 800 tonnes of wheat, while total barley production in the 25 states of the EU was 52 955 370 tonnes. Considering that the total cereal

production in Sweden was 5 050 600 tonnes in 2005, barley represents an important agricultural crop (FAOSTAT, 2006). Spring barley is an important source of animal feed, along with other cereals like oats or rye, and can constitute a good crop option with low running costs. Thus barley cultivation could be a source of income to farmers without placing high demands on inputs.

### *Weeds*

There are several definitions of weeds, depending on how human activities are affected by this kind of plant. Weeds can be considered as plants that compete with Man for possession of the soil, or plants that grow outside their place, or where they are not wished. They are also plants that invade crop cultures and are difficult to eliminate. They are not necessarily always small plants that affect crops, sometimes they are trees that invade natural communities.

When the soil is worked and seed sown, a regeneration process takes in the ecosystem. The physiological characteristics of some plants allow them to grow better in bare soil. These plants could be called weeds, since they colonise the soil before the crop and therefore interfere with agricultural practices and may cause damage in different ways. Concerning the crop, the main disadvantage is that weeds compete against it for growth factors such as nutrients, water, space and light. In addition, in many cases weeds have similar life cycles to cultivated plants and because of this, weed-crop competition is increased. Furthermore, weeds can also increase the risk of pests in the crop, since they act as hosts to insects and diseases in periods when no crop is present.

One of the main consequences of this competition or host action tends to be a decrease in crop yield. Poor weed control in cereals can lead to considerable yield losses, make harvesting difficult and is visually undesirable. In addition, poor control in one season can lead to problems with increased weed populations in subsequent crops (Teagasc, 2004).

The presence of weeds does not always pose a problem from an economic point of view. Small amounts of weeds in a culture can usually be tolerated, especially since the cost of eliminating them can be greater than the advantages of any marginal increase in yield arising from their elimination. In some cases, the presence of weeds can even produce a positive effect for the agriculture resource, since they can act as feed and shelter for many natural enemies to pests. Weeds also provide soil cover, protecting the surface from erosion action. In addition, root growth helps to improve biological soil activity and the structure of the soil. Therefore in an ecological perspective, the consequences of reducing weed populations could sometimes be very aggressive for the soil.

Current agricultural practices include the removal of weeds from within and around crops (Povedaa *et al.*, 2006). Mechanical elimination is a common method, since it is quite effective for perennial weeds as well as those that germinate on the surface. However, the use of mechanical techniques in the long term causes the loss of the soil vegetative cover and this can bring about serious damage to the soil. The other usual method of removal consists of application of chemical compounds over weed spots or in some cases directly over the ground, in fact herbicides are the most commonly used crop protection chemicals worldwide. However, the continued use of these chemicals in the long run brings about herbicide resistance and so induces the spread of new weed species that become

prevalent. This leads to increased weed control problems and also jeopardises the environment by chemical pollution.

In general, all the traditional techniques aim for total removal of weeds, leaving the surface between rows and lines almost completely bare. The question is whether there is any possibility of getting an economically acceptable weed level, while avoiding the use of such extreme techniques. Perhaps the answer can be found in nature itself and the natural balance between plant populations. The competition or interaction relationships that exist naturally could play an important role in solving this problem. The emergence period for the weed flora in relation to the crop can determine the competition intensity between crop and weed. Therefore this period of time can be the key to getting a good balance between them. Weeds that emerge after approximately one third of the life cycle of the crops do not usually modify the final crop yield. Otherwise, the yield losses caused by weeds can vary enormously depending on other diverse factors: the species of weeds and of the crop, their respective densities, the duration of the period of competition, the climatic conditions during the year, the characteristics of the soil, *etc.*, and all these aspects should be taken into account.

#### *Plant communication*

Plants generate and receive informational signals. An interaction between plants is called informational when it involves the exchange of an insignificant amount of matter or energy, in quantitative terms, but in spite of this has a profound effect on plants by modulating their developmental programme (Aphalo *et al.*, 1999). Several studies about interactions between plants have been carried out, for instance how plants use light signals to detect neighbouring plants (Aphalo *et al.*, 1999), how volatile communication between barley plants affects biomass allocation (Ninkovic, 2003) or how volatiles help cereals to defend themselves against aphids (Pettersen *et al.*, 1996).

It is known that many plants, crops as well as plants existing in the natural flora, can interact with each other by emitting volatiles (Dicke & Van Loon, 2000). It is also known that plants produce secondary metabolites (allelochemicals) and these natural chemical compounds are emitted as volatile complexes thrown out by the leaves, but they can also be exuded through the roots or washed onwards by rainwater. This process is called allelopathy.

It has been demonstrated that these chemical compounds can sometimes act as phytotoxins, promoting some biological processes in other organisms or plants (Reigosa *et al.*, 1999). These substances produced by individual plants can cause various effects on neighbouring plants, not only promoting but also inhibiting their development, or affecting the germination of seeds or seed viability. In addition, these effects can be modified depending on the concentration of allelopathic substances exuded. It has been demonstrated *e.g.* that excessively high concentrations can cause a decrease in the growth of neighbouring plants, but that when such concentrations are diminished the effect is reversed, improving the growth of the competing plant. Therefore, although it is very difficult to separate from other types of process, allelopathy plays a role in the ecological relationships between species, and it is rather interesting to know that sometimes there are allelochemicals that are not normally produced if the plant is not under stress (Reigosa *et al.*, 1999).

Root exudation has been less studied, but it can be very important because it can have a direct effect on the roots of other plants (Robinson, 1972). Allelopathic interactions have been identified in all major temperate cereal crops, wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.) and rye (*Secale cereale* L.) (Lovett & Hout, 1995). For instance, barley has proved to be allelopathically active against some weed species, inhibiting seed germination and growth of selected plant species (Overland, 1966; Liu & Lovett, 1993). Barley plants are known to send out allelochemicals compounds through their roots. Neighbouring barley plants have in previous studies been seen to induce a defence mechanism against weeds when they exude allelochemicals, and thus those allelochemicals could be used as natural pesticides (Duke & Abbas, 1995).

### *Competition*

The competitive ability is the probability of winning in competition with another species in a particular environment (Lambers *et al.*, 1998). Aarssen (1983) proposed that competitive ability could be divided into exploitation competition and interference competition.

Lambers *et al.* (1998) interpreted interference competition as the competition which harms another in the process of seeking a resource, even if the resource is not in short supply. The plant traits that contribute to interference competition are for instance the release of substances that are toxic to other species.

Aarssen (1983) claimed that the coexistence of plants in the same niche is permitted because there are numerous possible permutations and combinations of biological attributes in plants, which are roughly equivalent in the overall competitive power that they confer. However, plants that live in the same place at the same time try to obtain the available resources. These resources can be reduced or exhausted and sometimes more difficult to access for some plants due to the presence of others. The interaction among organisms (of the same or different species) that utilise common resources that are in short supply is called resource competition or exploitation competition (Lambers *et al.*, 1998).

The competition is fiercer between species whose niches are similar, so it is rather important to know the weed and crop species, because the affinity for the ecological niche between two individuals of the same species is greater than that between individuals of different species.

Plant organisms compete for nutrients, space, light, organic material, susceptibility to pests or diseases, *etc.* More adapted plants grow better because they can get more energy from the ecosystem. The available water (soil humidity), nutrients and light are essential resources for cultivated plants. In dry soil, the key factor in competition is water, something that is not so vital in moist soil. In agroecosystems, the competition for light has been reported to be one of the prime factors and the parameters for light utilisation to be among the best predictors of competitive success (Zimdahl, 1980; Holt & Orcutt, 1991; Blackshaw, 1994; Holt, 1995; Didon, 2002). Plant density is another factor to take into account, since when it increases, the competition for resources also increases, and therefore in parallel with increased weed density, the yields of the crop diminish progressively.

# Materials and Methods

## Experimental design

This study was carried out at the Swedish University of Agricultural Sciences (SLU) between March and April 2006, in a greenhouse with three two-rowed spring barley (*Hordeum vulgare* L. spp. *vulgare*) cultivars (Henni, Hydrogen and Troon). These three barley cultivars were grown as pure stands, all possible two-cultivar mixtures and the three-cultivar mixture. The proportions of the cultivar in the mixtures were 33:33:33 in the three-mixture cultivar, and 50:50 in the mixture of two cultivars. These barley cultivars were chosen on the basis of different data (Table 1) because they differ in three characteristics, allelopathic activity, root length development, and shoot length in the first growth stages. Two weed species, turnip rape (*Brassica rapa* cv. Agat) and perennial ryegrass (*Lolium perenne* cv. Helmer), were chosen as model weed flora. The barley plants were cultivated in plastic boxes (35 cm x 45 cm x 23 cm deep, surface area 0.16 m<sup>2</sup>) with or without weeds. The experiment comprised a total of 60 boxes and was arranged as completely randomised blocks, which consisted of 15 treatments with four replicates. The glasshouse area was divided into 4 blocks and the boxes were moved within the block once a week to counteract border effects and differences in temperature and light intensity between different parts of the greenhouse.

The soil used in the experiment was a sieved sandy loam soil taken from Ultuna, Uppsala. The boxes were filled with 20 cm depth of soil, *i.e.* to within 3 cm from the top. The upper 3 cm in the boxes consisted of a weed-free soil in order to reduce the naturally occurring weed flora (Hasselfors Garden, Hasselfors, Sweden). Spring barley was sown at a rate of 231 viable barley grains m<sup>-2</sup>. In the boxes with barley there were 7 barley rows with 4.8 cm spacing between rows and 17 kernels row<sup>-1</sup>. Immediately afterwards, a soil layer of 2.5 cm was added to all the boxes. Weeds were sown on the same day as barley. Turnip rape seeds were oversown 2.5 cm higher between the rows of barley at a rate of 313 turnip rape seeds m<sup>-2</sup> with 2 cm spacing between rows and 50 seeds rows<sup>-1</sup>, while the ryegrass was sown in the same seedbed but was broadcast randomly. The sowing density was 5 g ryegrass seeds m<sup>-2</sup>. Finally, a layer of 0.5 cm of soil was added to all the boxes. Two weeks after sowing the number of barley plants was reduced to a uniform density of 480 plants m<sup>-2</sup> (77 plants box<sup>-1</sup>). All the boxes were watered to leave the soil moisture level close to field capacity. During all the experiments, water was supplied depending on plant needs, which changed during the growth stages. Along with the water a nutrient solution corresponding to 1 mL Blomstra L<sup>-1</sup> water (Blomstra: 5 g N, 1 g P and 4.3 g K 100 mL<sup>-1</sup>) was applied. The temperature in the greenhouse was 18 ± 2 °C by day and 10 ± 2 °C by night. The light period was 18 h day<sup>-1</sup>, which was achieved by artificial lighting automatically started as the sunlight went below 200 W m<sup>-2</sup>. There was no need for any pest control measures.

Table 1. *Characteristics of the selected barley cultivars Henni, Hydrogen and Troon*

Cultivar characteristics	Year	Henni	Hydrogen	Troon
Allelopathy <sup>(1)</sup>		High	High	Low
Shoot length <sup>(1)</sup>		Medium	Medium	High
Root length <sup>(1)</sup>		High	Medium	Medium
Straw length at ear emergence <sup>(2)</sup> (cm)	2002	57	58	63
	2003	57	53	58
Emergence date <sup>(2)</sup>		2304	2304	2306
Grain yield <sup>(2)</sup>	2002	43.8	48.1	49.6
	2003	47.8	56.9	58.3
% Weed cover <sup>(2)</sup>	2002	13	7	12
	2003	-	13	-
	2004	-	24	-

<sup>(1)</sup>Allelopathy, shoot and root length characteristics, Nils-Ove Bertholdsson Svalöv Weibull AB

<sup>(2)</sup> Means from FØJO BAR-OF, database from ecological farming experiments in Denmark ([www.planetinfo.dk](http://www.planetinfo.dk))

## Measurements

The main shoot length was recorded throughout the experiment. Ten barley plants from each box were randomly taken for these measurements. The number of turnip rape leaves at 27 days after sowing (DAS) and the different flower stages at 27 and 31 DAS were measured in ten plants selected at random. The harvest was taken at the end of the experiment during the beginning of the ear emergence stage, at 49 DAS. At harvest, 10 plants of barley and turnip rape respectively and 20 plants of the ryegrass plants were chosen at random from each box and cut at ground level. In the 10 barley plants, the development stage was recorded as regards decimal code scale (Zadoks *et al.*, 1974; Tottman, 1987). In the selected plants of barley and ryegrass, the numbers of tillers were counted and the length of the main shoot was measured. In the selected 10 plants of turnip rape, the length and diameter of the main shoot were measured, and the vegetative and reproductive parts were weighed separately. All these selected plants were weighed fresh, dried at 105°C for 24 h and weighed again. The remaining barley, ryegrass and turnip rape plants were counted, cut at ground level, weighed fresh, dried at 105°C for 24 h and weighed again.

## Calculations and statistics

In order to determine the effectiveness of the mixtures as a weed control, two calculations were carried out:

1) Competitive effect (CE) of barley on weed biomass, which represents the ability of the barley to suppress weeds. This parameter was calculated using the equation 1:

$$CE = 1 - (Z_B / Z) \quad [1]$$

where  $Z_B$  and  $Z$  are the biomass when the weeds were grown with and without the barley cultivars, respectively.

2) Competitive response (CR) of crop biomass, which represents the ability of the barley to tolerate being suppressed by weeds. This was calculated using equation 2:

$$CR = 1 - (Y_W / Y) \quad [2]$$

where  $Y_W$  and  $Y$  are the biomass when the barley cultivars were grown with and without weeds, respectively.

In addition, CE was calculated as the effect on number of leaves in turnip rape plants, and CR was calculated as the effect on shoot length in barley plants.

Data from the measurements were analysed statistically using the SAS statistical package (SAS 8.0, SAS Institute Inc). The analyses of variance were conducted according to the experimental designs employed and treatment means were compared by least significant difference at the 5% level of probability. All statements in the Results section on treatment responses are statistically significant ( $p < 0.05$ ) less otherwise stated.

## Results

### Weed flora

#### *Turnip rape*

The dry weight and number of leaves of turnip rape were affected when grown in competition with barley. These parameters overlapped, meaning that when the biomass was reduced, the number of leaves was also reduced. For example, the mixture with Hydrogen and Troon, which had the lowest competitive effect (CE) of barley on the number of leaves on turnip rape at 27 days after sowing (DAS), also had the lowest CE on total turnip rape biomass at 49 DAS (Figure 1). This mixture had 13% lower CE on dry weight than the Hydrogen and Henni mixture and 17% lower CE on dry weight than Hydrogen grown in pure stand. The Henni and Troon mixture had the second lowest CE in both parameters.

Turnip rape produced 74% lower biomass when grown in competition with ryegrass and the barley mixture of Hydrogen and Troon cultivars compared with when grown in competition with ryegrass and with Hydrogen in pure stands (Table 2).

The fresh biomass of turnip rape flower at harvest (49 DAS) was four times higher when grown in competition with Hydrogen and 2.5 times higher when grown with the mixture Henni and Troon than when grown in competition with the mixture Hydrogen and Troon (Table 2).

None of the parameters flower stage, shoot length, shoot diameter, fresh weight, fresh weight of vegetative parts and individual dry weight measured at 49 DAS (harvest) showed changes when turnip rape plants were grown with all combinations of the three barley cultivars.

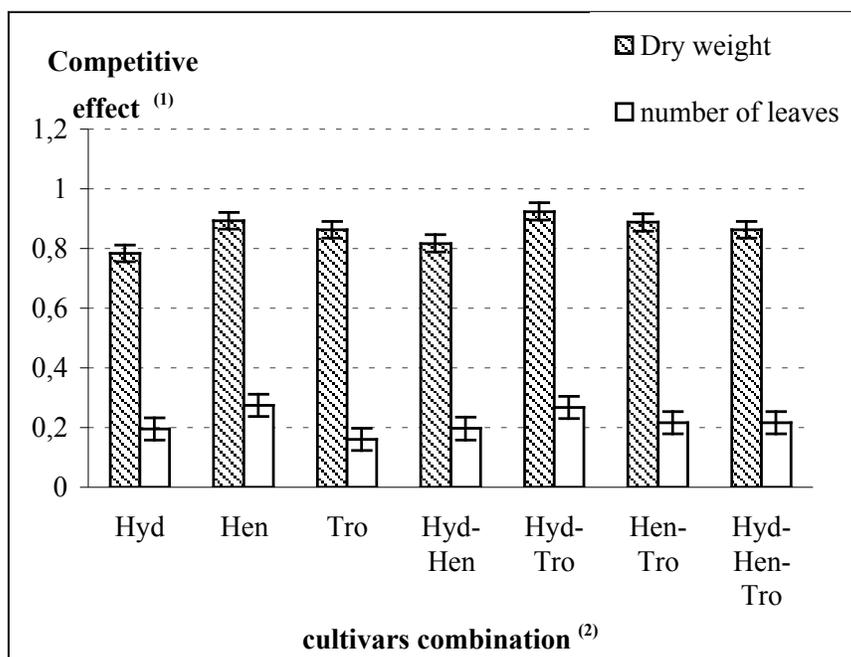


Figure 1. The competitive effect of three barley cultivars (*Hordeum vulgare* L. cvs. Henni, Hydrogen and Troon) on the number of leaves on turnip rape (*Brassica rapa* L.) at 26 DAS and on the total aboveground dry matter (DM) biomass at 49 DAS. Vertical bars represent  $\pm$  standard error. Positive values indicate negative effects of barley on turnip rape, the inhibition of biomass production or number of leaves.

Table 2. Aboveground biomass of turnip rape plants at 49 DAS in relation to the 8 different barley cultivar combinations tested

CULTIVAR COMBINATION	BIOMASS	
	Fresh matter <sup>(1)</sup> of reproductive parts (g)	DM <sup>(2)</sup> of vegetative parts (g)
Hyd	22.17 a	18.9 b
Hen	8.82 bc	9.3 bc
Tro	10.07 bc	11.47 bc
Hyd - Hen	17.72 ab	16.85 bc
Hyd - Tro	5.57 c	6.97 c
Hen-Tro	9.32 bc	9.62 bc
Hyd-Hen-Tro	13.27 abc	12 bc
Without barley	-	92.37 a

<sup>(1)</sup> Means within columns followed by different letters are significantly different (p<0.05).

<sup>(2)</sup> Means within columns followed by different letters are significantly different (p< 0.001).

### Ryegrass

The total DM production of ryegrass (*Lolium perenne* L.) was 9-27% lower when ryegrass was grown in competition with barley than when grown with only turnip rape plants (Table 3). In general, the biomass (DM) of ryegrass did not respond to the different cultivar combinations, the response only varied between two barley mixtures. In the presence of cvs. Hydrogen and Troon in mixture, the dry weight of ryegrass had the lowest reduction (44%) compared with when grown with the three-cultivar mixture, where the reduction in ryegrass biomass was 72%.

No changes in fresh weight, shoot length, number of tillers and number of plants measured at 49 DAS were seen between the treatments. There were also no differences regarding the competitive effect on ryegrass in DM production.

Table 3. Aboveground DM of ryegrass (*Lolium perenne* L.) plants when grown together with turnip rape (*Brassica rapa* L.) and with or without barley, respectively. Measured after harvesting (49 DAS), in relation to the 8 different barley cultivar combinations tested

<i>CULTIVAR COMBINATION</i>	<i>BIOMASS</i> Dry matter (g)
Hyd	1.73 bc
Hen	1.88 bc
Tro	1.88 bc
Hyd - Hen	1.45 bc
Hyd -Tro	2.35 b
Hen - Tro	1.40 bc
Hyd - Hen - Tro	1.15 c
Without barley	4.25 a

Means within columns followed by different letters are significantly different ( $p < 0.05$ ).

#### *Total weed flora*

When the Competitive Effect (CE) of the crop on the total weed flora biomass was analysed, the dry weight of weed plants was affected when grown in competition with barley. The mixture of Hydrogen and Troon had the highest CE of barley on weed biomass (Figure 2), with a 12% effect than the Hydrogen and Henni mixture and a 16% higher CE on dry weight than Hydrogen grown in pure stand. These CE of the barley cultivar mixtures on the total weed flora did not differ from the CE on the turnip rape.

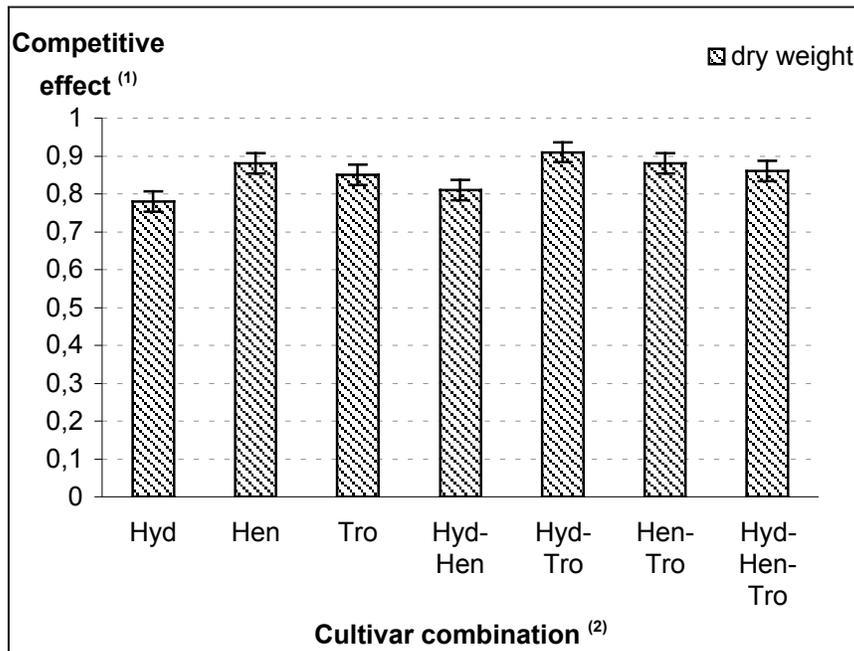


Figure 2. The competitive effect of the three barley cultivars tested on the model weed flora total aboveground DM biomass at 49 DAS. Vertical bars represent  $\pm$  standard error. Positive values indicate negative effects of barley on weeds, the inhibition of biomass production.

### Barley cultivars

None of the morphological parameters of barley, such as number of tillers, number of leaves, number of plants, showed changes when barley cultivars were grown with weeds. The development stage measured at harvest also showed no differences.

#### *Dry matter production*

In competition with weeds, when cv. Hydrogen was grown in a mixture with Troon and with Troon and Henni, the barley biomass produced was 32% and 34% higher, respectively, compared with when cultivated in pure stand (Table 4). However, the mixture of the cultivars Hydrogen and Henni in competition with weeds did not produce a change in DM production compared with Hydrogen in pure stand. No differences were found in the total biomass produced by the combination of Henni and Troon cultivars compared with their production in pure stand when grown with or without weeds, respectively.

#### *Shoot length development*

At 21 DAS, the mixture Henni and Troon had 5% taller plants than cv. Henni in pure stand when grown in competition with weeds. However, this mixture did not produce any change in shoot length compared with Troon plants grown in pure

stand (Table 4). However cv. Troon in pure stand had 7% taller plants compared with the plants in the mixture Hydrogen and Troon, and also 6% taller than the plants in the three-cultivar mixture.

When barley was grown without weeds, the mixture Hydrogen and Henni developed 7% shorter plants than the plants of cv. Hydrogen grown in pure stand (Table 4). On the other hand, cv. Henni produced 6% shorter plants compared with the plants in the mixture of Hydrogen, Henni and Troon. However the Troon cultivar in pure stand did not show different shoot length development compared with the possible mixtures with this cultivar when grown without weeds.

When cvs. Hydrogen and Henni were grown in the presence of weeds in pure stand, they developed a stem 5% and 4% shorter, respectively, than when they were grown without weeds. However, when these cultivars were grown in a mixture, their behaviour was the opposite. Competing with weeds this mixture developed plants with 3% taller plants than when they were grown without weeds. In the three-cultivar mixture, the shoot length of barley plants showed a 7% reduction when barley was competing with weeds compared with when grown without them.

Table 4. Aboveground biomass DM and shoot length of barley cultivars grown with or without weeds

CULTIVAR COMBINATION	DM (g) <sup>(1)</sup>		Shoot length (cm) <sup>(2)</sup>	
	Without	With	Without	With
Hyd	79.97d	71.625d	42.54abcd	40.46ef
Hen	164.90a	109.03bc	41.1775cdef	39.365f
Tro	167.23a	111.47bc	42.8933abc	43.4675ab
Hyd - Hen	108.13bc	90.275cd	39.495f	41.1925cdef
Hyd - Tro	113.27bc	104.93bc	41.6133bcde	40.29ef
Hen-Tro	158.87a	114.25b	41.0233cdef	41.5925bcde
Hyd-Hen-Tro	127.63b	109.63bc	44.095a	40.8075def

<sup>(1)</sup> Dry matter biomass of barley cultivars at 49 DAS. Means followed by different letters are significantly different (p<0.001).

<sup>(2)</sup> Shoot length at 21 DAS. Means followed by different letters are significantly different (p<0.001).

#### *Number of leaves*

Barley plants tended to reduce the number of leaves and also to diminish the length of the main shoot when they were grown with weeds, regardless of whether they constituted a mixture of cultivars or whether they were grown in pure stand (Table 5). No interactions between the factors cultivar x weed were found, meaning that no differences between the cultivars were found in the number of leaves as affected by the weed flora.

#### *Competitive response*

No significant changes in biomass production were seen in Hydrogen in pure stand or in the Hydrogen and Troon mixture when they were grown with weeds

compared to without weeds. Therefore the cv. Hydrogen in pure stand and the Hydrogen and Troon mixture had a low competitive response (CR) on biomass (Fig. 3). Hydrogen in pure stand had a 77-79% lower CR than cvs. Henni and Troon in pure stands ( $p = 0.0548$ ). The mixture of all three cultivars had a 58% lower CR than cv. Troon in pure stand. The mixture Henni and Troon had a higher CR than Troon and Henni in pure stand. This mixture lost 29% of biomass when it was grown with weeds.

Concerning the shoot length, the two mixtures with the Henni cultivar showed a stimulation of the length in competition with weeds. The mixture of Henni and Hydrogen stimulated the stem growth by 4%, but on the contrary the three-cultivar mixture presented the highest reduction in length, 7%. Likewise, cv. Hydrogen had a negative CR on shoot length (Fig. 4).

The CR on number of leaves of barley plants did not show differences among treatments.

Table 5. *Variations in growth parameters when barley plants were grown with weeds*

WEED	VARIABLES			
	Number of leaves <sup>(1)</sup>		Shoot length <sup>(2)</sup> (cm)	
With	3.69	a	2.22	b
Without	4.00	b	2.27	a

<sup>(1)</sup> Average of number of leaves measured on barley plants at 21 DAS;

<sup>(2)</sup> Length (cm) of the stem of barley cultivar plants measured at 49 DAS.

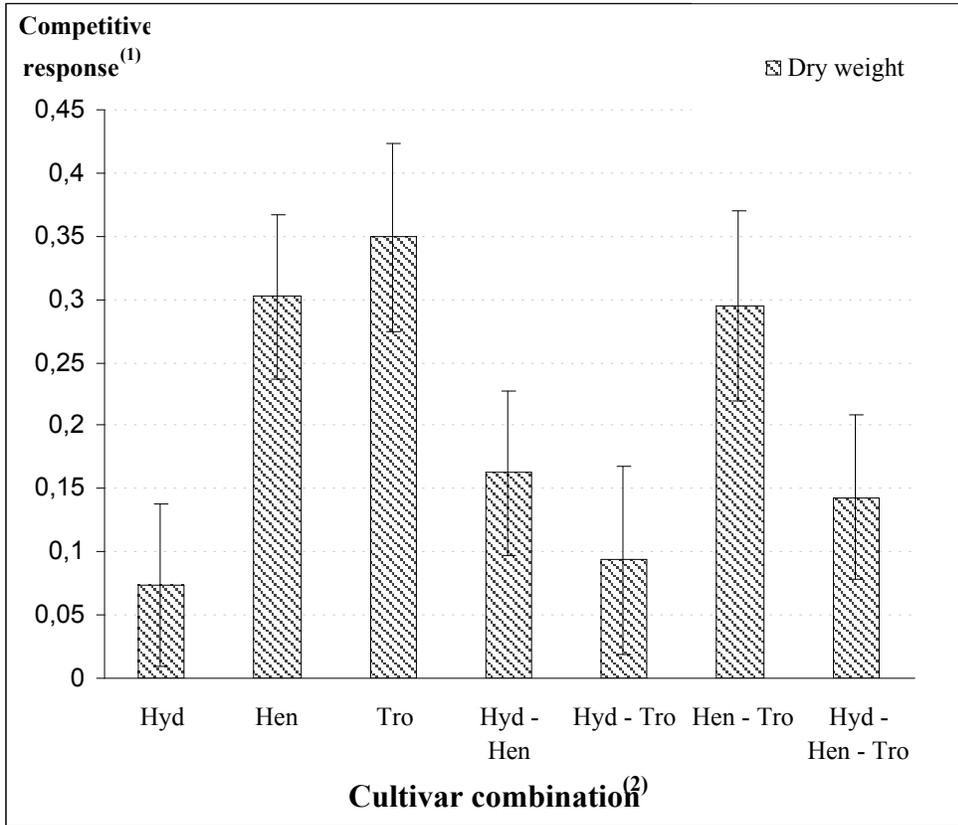
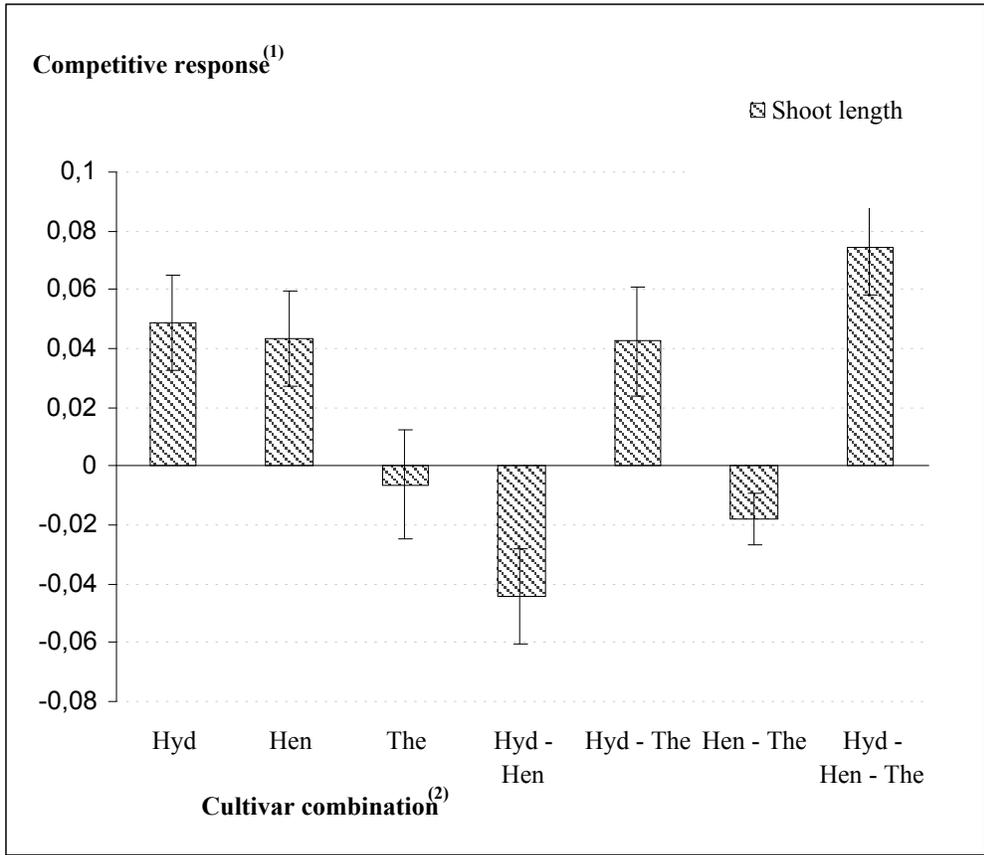


Figure 3. The competitive response in terms of total above-ground biomass DM at 49 DAS for the three barley cultivars grown with or without the model weed flora. Vertical bars represent  $\pm$  standard error. Positive values indicate negative effects of weeds on crop, the inhibition of biomass production. Negative values indicate positive effects of weeds on crop, the stimulation of biomass production.



*Figure 4. .* The competitive response in terms of shoot length at 49 DAS for the three barley cultivars grown with or without the model weed flora. Vertical bars represent  $\pm$  standard error. Positive values indicate negative effects of weeds on crop, the inhibition of shoot extension. Negative values indicate positive effects of weeds on crop, the stimulation of shoot extension.

## Discussion

In the experiment, there was a tendency for barley plants to reduce some growth parameters (number of leaves, shoot length) when they were grown with weeds, regardless of whether they constituted a mixture or not. The presence of weeds produced a change in behaviour, but although there was a growth reduction of the morphological parameters in the experiment, in other research an increase in these parameters has been seen. In fact Didon (2002) reported a stem length increase in some cases when barley cultivars competed with weeds. Therefore the kind of change in morphological parameters of barley plants when grown in competition with weeds depends on the characteristics of the barley cultivars.

## **Cultivar mixtures**

Weeds are a very wide concept and represent a group of plants in general. In this experiment, the model weeds used were a mixture of two different species, turnip rape and ryegrass. These species were chosen to represent totally diverse morphological and physiological characteristics. For instance when the Competitive Effect (CE) on weeds was analysed, the average amounts of biomass produced by weeds were significantly similar to the turnip rape results. This could be due to the bigger turnip rape size in comparison with ryegrass, so each one should be analysed separately. The differences in weed suppressive ability between the barley cultivar mixtures were not constant over the two different weed species. The mixture of the three cultivars was shown to have higher competitive ability against ryegrass dry matter than the Hydrogen and Troon mixture. On the other hand, the Hydrogen-Troon mixture was better for controlling turnip rape growth, since it showed a greater CE on dry matter biomass in comparison with the three-cultivar mixture.

The cultivars differed in competitive ability against weeds but mixtures improved on the ability of the cultivars in pure stands in some cases. Comparing the percentage turnip rape biomass reduction produced by each barley cultivar grown in pure stand and the biomass reduction in the mixture, the effect of the mixture in each case tended to be unaltered or even positive.

On the other hand, analysing the losses of biomass that barley presented when grown in competition with weeds compared with when grown without weeds, the conclusion was similar. The percentage barley biomass reduction shown by each cultivar grown in pure stand with weeds was similar but sometimes higher than the biomass reduction of the barley mixtures. It was only in the case of cv. Hydrogen that the mixtures with this cultivar produced a higher loss of biomass compared with the losses of cv. Hydrogen in pure stand. However this cultivar had a low biomass loss in competition with weeds overall compared to the other cultivars.

## **How to design a mixture**

This study showed that different cultivar mixtures behaved in distinct way with unequal competitive ability against weeds. Some mixtures demonstrated greater competitive ability compared with the cultivars in pure stands.

The Competitive Effect (CE) of barley on turnip rape biomass and number of leaves overlapped, so that when the biomass was reduced, the number of leaves was also reduced. However, barley competition can influence several weed aspects, as reproductive and vegetative development, in different ways. In this case there was also a correlation with the suppression of vegetative and reproductive parts. For instance, the Hydrogen and Troon mixture produced a low fresh weight of turnip rape flowers and also a high CE for vegetative parameters. Likewise, the Hydrogen cultivar, which showed one of the lowest CE on vegetative aspects, had also one of the highest flower biomass productions.

On the other hand, one of the mixtures with high competitive ability (Hydrogen-Troon), did not contain the cultivar with best CE for vegetative parameters in pure stands, which was in fact Henni. However, when this Henni cv. was added to the

Hydrogen-Troon mixture, there was no positive effect and the CE with this three-cultivar mixture was lower.

The Hydrogen cultivar in pure stand presented the lowest Competitive Response (CR) in terms of dry matter production. This means that it grows with weeds without great loss of biomass, perhaps due to its high allelopathic capacity. In terms of biomass losses, cvs. Troon and Henni in pure stand did not compete as effectively as cv. Hydrogen against weeds. Troon is characterised by its long stem and Henni by its deep roots, and therefore these characteristics seem to not be as effective as allelopathy capacity. The mixture of these cultivars also presented a high CR in terms of barley dry matter, but all the other possible mixtures showed an improved CR for dry matter, *i.e.* lower losses compared with the behaviour of each mixture component in pure stand. For this reason cv. Hydrogen could play an important role in this aspect, because only the mixtures with this component exhibited this positive change.

The CR of barley cvs. in reference to shoot length was totally different compared with the CR of barley on dry matter. In the case of shoot length, the Hydrogen-Henni mixture showed the best behaviour, with a 4% stimulation of shoot length when competing with weeds. However, none of the components of this mixture had a stimulation of the stem length when grown in pure stand, and the best CR on pure stands was unexpectedly shown by cv. Troon, which is stimulated to shoot extension when grown with weeds. Likewise, the combinations of Troon-Hydrogen and Troon-Hydrogen-Henni produced an inhibition of shoot length (great CR), so the positive effect of Troon cv. was not expressed within these combinations. Cv. Henni in combination with Hydrogen and with Troon produced the best CR on shoot length options, but this cv. Henni showed one of the greatest CR in pure stand. Therefore CR in terms of shoot length did not show any relationship between the behaviour of cultivars in pure stands and within a mixture that could explain the improvement shown by the mixtures, as happened in CR of dry matter.

Analysing the characteristics of each barley cultivar individually, it was not possible to deduce that mixture effects are the sum of these. This means that if cultivars with high values of allelochemicals (cv. Hydrogen) and cultivars with long stem growth (cv. Troon) are mixed, the mixture will not have the sum of these two characteristics, or rather, it will not have the sum of the effects that these characteristics produce on weeds in pure stands.

In conclusion, mixture design is the key to success in mixing cultivars that can act as weed control. I could not find a standard conduct of these barley cultivars within the mixture, and the analysis of the main characteristics of these cultivars, namely allelopathy, long shoot and long roots, was not sufficient to explain the behaviour of the mixtures. Deeper study of the cultivar characteristics and intraspecific relationships between cultivars could help to achieve a proper mixture design.

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## Appendix: Photos



**Appendix 1 figure 1.** *Hordeum vulgare* spp. vulgaris cultivar Hydrogen at 34DAS (days after sowing) grown in pure stand (without weeds).



**Appendix 1 figure 2.** *Hordeum vulgare* spp. vulgaris cultivar Henni at 34DAS (days after sowing) grown in pure stand (without weeds).



**Appendix 1 figure 3.** *Hordeum vulgare* spp. vulgaris cultivar Troon at 34DAS (days after sowing) grown in pure stand (without weeds).



**Appendix 1 figure 4.** Three barley (*Hordeum vulgare*) cultivar mixture at 34DAS (days after sowing) grown in pure stand (without weeds).



**Appendix 1 figure 5.** *Hordeum vulgare* spp. vulgaris cultivar Hydrogen at 34DAS (days after sowing) grown in competition with weeds.



**Appendix 1 figure 7.** *Hordeum vulgare* spp. vulgaris cultivar Troon at 34DAS (days after sowing) grown in competition with weeds.



**Appendix 1 figure 6.** *Hordeum vulgare* spp. vulgaris cultivar Henni at 34DAS (days after sowing) grown in competition with weeds.



**Appendix 1 figure 8.** Three barley (*Hordeum vulgare* spp. vulgaris) cultivar mixture at 34DAS (days after sowing) grown in competition with weeds.



**Appendix 1 figure 9.** Two barley (*Hordeum vulgare* spp. vulgaris) cultivar mixture Hydrogen-Henni at 34DAS (days after sowing) grown in competition with weeds.



**Appendix 1 figure 10.** Two barley (*Hordeum vulgare* spp. vulgaris) cultivar mixture Hydrogen-Troon at 34DAS (days after sowing) grown in competition with weeds.



**Appendix 1 figure 11.** Two barley (*Hordeum vulgare* spp. *vulgare*) cultivar mixture Troon-Henni at 34DAS (days after sowing) grown in competition with weeds.