Beware of your neighbour

– The effects of intraspecific interactions and CO_2-level on the plant growth of two Barley cultivars

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Abstract

Plants have evolved several ways to sense what is going on around them. They can detect possible competitors by picking up on airborne Volatile Compounds emitted from neighbouring plants. The cues can induce growth responses that enhances the competitive ability of the receiver. Typically, plants are bred for the purpose of growing in pure stands at high densities with only the same cultivar. However, recent research has shown that some cultivars benefit from growing in a cultivar mixture. It is important to raise the knowledge about how specific cultivars respond to different kinds of competition since it can help growers to better customize the cropping system to fit the needs of the cultivar in question. Another aspect of this study concerns CO_2. With a changing climate, the level of atmospheric CO_2 will increase. Elevated levels of CO_2 have been shown to increase the rate of photosynthesis in C3 plants resulting in increased plant growth. But there is currently a lack of knowledge regarding how elevated levels of CO_2 effects the interactions between plants. The aim of this study was to examine how two cultivars of Barley change their growth pattern when they are exposed to different forms of intraspecific competition and if changing levels of CO_2 will influence the responses. In chambers with controlled conditions, some plants grew in a cultivar mixture, whilst other grew in pure stands. Two different treatments of CO_2 were used to see how elevated levels of CO_2 would impact the intraspecific interactions. No difference was found between the two forms of competition on the plant growth of barley and since no difference was found between the groups no conclusions could be drawn regarding the effects of elevated levels of CO_2 beyond that it induced overall plant growth. However, the data indicated that different cultivars exhibit unique growth rates and responses when exposed to different forms of intraspecific competition. There was also an indication that elevated levels of CO_2 effect the competitive ability of different cultivars to varying degrees.

Keywords: Barley, ‘Salome’, ‘Fairytale’, Cultivar mixture, Pure stand, Intraspecific competition, Plant-plant interactions, Competitive ability, Elevated CO_2

Table of contents

List of tables and figures

1. Introduction

1.1. Environmental cues

The environmental conditions surrounding a plant are ever changing. On a daily basis, plants are faced with a wide range of both abiotic and biotic stresses (Suzuki et al. 2014). There are fluctuations in temperature, changes in light-availability and different amounts of resources in the soil. Furthermore, plants are constantly competing with neighbouring plants and must be able to defend themselves from a wide range of herbivores and pathogens (Rejeb et al. 2014). To survive in the complex web that makes up the habitat of a plant, its essential to listen in on environmental cues (Lamers et al. 2020). Since plants are rooted in the ground and cannot move around, they need other strategies that lets them know what is going on around them so they can respond accordingly. Plants have evolved several ways to sense their surroundings. They have been shown to respond to touch, light, gravity and even to sounds (Braam 2005; Holt 1995; Vandenbrink & Kiss 2019; Khait et al. 2019). One environmental cue, that plants can sense, is that of airborne chemicals called Volatile Organic Compounds (VOCs) (Brosset & Blande 2022). VOCs are gasses or smells that are emitted from plants (Picazo-Aragonés et al. 2020). When VOCs are mentioned in this text, it refers to airborne volatile compounds emitted from aboveground plant tissue and not belowground VOCs emitted from roots.

1.2. Function of VOCs

The release of VOCs has three main functions in plants. To start off, VOCs act as a defence mechanism against both biotic and abiotic stress. VOCs also work as a signal for pollinators, and they are involved in the interactions between neighbouring plants (Maffei et al. 2011). In plant-plant interactions, VOCs are info-chemicals that carry information to a receiver about the conditions of the sender. For instance, an old leaf with necrotic spots emits a different mix of VOCs than that of a healthy leaf, conveying different messages regarding if the leaf is edible or not

(Pierik et al. 2013). If a plant picks up on the volatile chemicals sent out by injured plants in its surroundings it becomes primed to raise its defences and can prepare for a pending attack (Pierik et al. 2013). In this text, the focus will be on the interactions between neighbouring plants.

1.3. Smell the competition

Plants are both senders and receivers of VOCs and pick up on chemicals that neighbouring plants send out (Sugimoto et al. 2016). In nature, coexistence is an undeniable fact in the life of a plant. Plants are coexisting with the surrounding vegetation and neighbouring plants are forced to share available resources. However, when resources are slim, plants must compete if they want to survive. Essentially, they are competing for space. Plants are bound to a certain soil volume and the vertical space is limited in the sense that it is not viable to keep reaching upwards endlessly (Craine & Dybzinski 2013). By closely monitoring the competition and by picking up on kairomones emitted from competitors, plants can make appropriate responses that increase their fitness (Ninkovic et al. 2016). Even though plants affect their surroundings and can make it unattractive or harder for possible neighbours to establish themselves, they cannot freely choose their neighbours. However, different strategies help a plant to get ahead of the competition (Novoplansky 2009). For instance, they can focus more energy into expanding the root system to reach more nutrients and water, or focus more on elongation or increasing the area of the leaves to avoid getting shaded or to monopolize more of the available light (Craine & Dybzinski 2013).

1.4. Stranger or kin

Plant-plant interactions can be both intraspecific, meaning that plants of the same species interact and affect each other, as well as interspecific, meaning that the plant interacts with and is affected by members of another species. Being able to detect if your neighbour is a kin or a stranger is an important trait since it can determine which response that is most beneficial (Ninkovic et al. 2016). Two cultivars of the same species are genetically kin. However, since they have been bred to possess unique characteristics and traits they can be seen as strangers. In this trial two cultivars of Barley were used, ‘Salome’ and ‘Fairytale’. Since they are the same species, the competition that occurs between them is intraspecific, regardless of if they grow together in a cultivar mixture or by themselves in a pure stand. Higher diversity within a plant community has been shown to promote productivity due to different resource demands (Reusch et al. 2005; Zuppinger-Dingley et al. 2014). The yield of barley was increased when different cultivars grew together in a mixture compared to when they grew in pure stands with only the same species (Essah & Stoskopf 2002). However, this was not the case for all combinations tested. Similarly, increased productivity was found in a diverse grassland with higher levels of interspecific interactions (Zhang & Wang 2011).

1.5. Circumstances determine responses

Depending on the circumstances the effects of plant-plant interactions can differ. One response mechanism in plants is that they allocate biomass to different parts to alter their growth. This was prevalent on two cultivars of Barley. When the cultivar 'Kara' was exposed to VOCs from the cultivar 'Alva', it allocated biomass to the roots (Ninkovic 2003). The opposite was found in another trial examining the responses of the same two cultivars of barley. Instead, the receiving plant focused on allocating biomass to elongation to avoid getting shaded (Kegge et al., 2015). All though it wasn't the case for all combinations tested. Nonetheless, this points towards responses being context dependent. If some circumstances change the responses can vary. The effects of plant-plant interactions differ depending on (i) the plants in question, and (ii) which context it is (Conrath et al. 2015). For instance, the negative effects that intraspecific competition had on the yield and plant growth of barley could be somewhat mitigated by additional nitrogen fertilization (Kumar et al. 2020).

If circumstances change, so does the outcome and growers benefit from recognizing what factors that can induce certain responses. As a grower you have a limited space to grow your crops and there is always a trade-off between maximising the available area while at the same time not having your plants to close that they affect each other negatively. By understanding how specific cultivars affect and respond to each other it becomes easier to promote plant growth since cropping systems can be customized to fit the needs of the cultivar in question. Furthermore, plants have natural competition strategies and by knowing how these systems are induced or supressed they can be used as a tool to increase yields. In this trial, two forms of intraspecific competition are compared. Intraspecific competition occurring in a cultivar mixture with two different cultivars of barley and intraspecific competition occurring in pure stands with only the same cultivar.

1.6. A changing climate

Along with climate change, challenges arise for the agricultural sector. Drought-affected areas are projected to increase from 15 to 44 % by 2100. By 2050 the yield of major crops in drought areas are projected to decrease by a staggering 50 %

(Malhi et al. 2021). Extreme weather is increasing globally resulting in crop losses (Powell & Reinhard 2016) and the planets fresh water reservoirs are getting smaller (OECD 2016). By 2050, atmospheric CO_2 will reach a level up to 600 ppm and by 2100 the atmospheric CO_2 is projected to reach levels as far as up to 1000 ppm (Gutowski & Johns 2013). All these factors will impact the way agriculture is managed and changes will have to be made to ensure global food security. Nonetheless, elevated levels of CO_2 have been shown to increase the rate of photosynthesis in C3 plant, resulting in higher yields (Gardi et al. 2022). After examining 98 different genotypes of barley, statistical significance in aboveground biomass (AGB) was found when barley grew in 700 ppm compared to 400 ppm. Furthermore, 68 out of 98 of the genotypes responded positively to raised levels of CO_2 and had increased AGB whilst 30 did not (Mitterbauer et al. 2017). More than only increasing carbon fixation in leaves, elevated levels of CO_2 has also been shown to decrease the water use by 5-20 % (Taub 2010). In the same study however, it was found that protein as well as the nitrogen concentration of plant tissue decreased with higher levels of CO_2. CO_2 is not the only factor that will change in the future and when temperature is considered, the equation changes. In a comprehensive study on 138 cultivars of barley exposed to future predicted climatic conditions in an enclosed greenhouse, the overall yield decreased by 29 % (Ingvordsen et al. 2015). The control used was current climate conditions of southern parts of Scandinavia.

Although positive effects have been seen on plant growth and water use, research regarding how intraspecific competition between different cultivars is affected by elevated levels of CO_2 is slim. Soybean was shown to increased its competitive ability under elevated levels of CO_2. However, so did the weed lambsquarter whilst the weeds pigweed and millet had a decrease in their competitive ability (Miri et al. 2012). By understanding how the competitive ability of different cultivars is affected by changing levels of CO_2, the agricultural industry become better prepared to develop more sustainable agricultural practises for the future. Furthermore, it is a goal to increase the knowledge of plant function and how plants respond to changing environmental conditions. With a fuller picture of how plants work it will be easier to tackle future challenges arising from a changing climate.

1.7. Aim

The aim was to examine how two cultivars of Barley change their growth pattern when they are exposed to different forms of intraspecific competition and if changing levels of CO_2 will influence the responses.

1.7.1. Research questions

(i) Does intraspecific competition involving a cultivar mixture of barley lead to more plant growth compared to intraspecific competition in pure stands with barley?

(ii) Do elevated levels of CO_2 effect the intraspecific competition of barley?

2. Methodology

2.1. Study site and treatments

The study was conducted in the Biotron at Vegetum in Alnarp. Two chambers with controlled conditions where used. Both chambers had the same conditions when it came to light, relative humidity, and temperature (Table 1).

Table 1. Settings for light, temperature, humidity and CO_2-concentration of the two chambers used. The only different condition was the CO_2-concentration.

Chamber	CO_2	Temperature	rH	Light intensity	Photoperiod
1	400 ppm	26 °C	70.0 %	250 µ mol/m2/s	L: 12h, D: 12h
2	1000 ppm	26 °C	70.0 %	250 µ mol/m2/s	L: 12h, D: 12h

The only varying factor between the two chambers was the amount of CO_2 in the air. One of the chambers had a CO_2-concentration of 400 ppm, which is close to ambient conditions and served as a control. The other chamber had a concentration of 1000 ppm as a proxy for future increased levels of CO_2. Since the study took place inside, under controlled conditions, the trial was not time dependant and can be replicated throughout the year. One thing to note is that other trials took place in the same chambers during this specific time period, which means that there were more plants in the chambers then just the plants of this trial.

2.2. Arrangement and plant material

Two cultivars of barley *Hordeum vulgare* were used in the trial, 'Salome' and 'Fairytale'. 'Salome' is generally more fast growing then 'Fairytale'. To increase the sample size, two rounds of the same trial was run. However, some modifications were made for the second trial. The first trial ran for two weeks, and the second trial ran for three weeks. The plants were

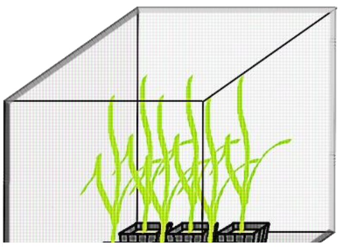

Figure 1. Experimental setup. Six plants per bug dorm in separate pots.

placed into plastic bug dorms with six plants per box (Figure 1).

Figure 2. Shelf arrangement.

The lid and bottom of the dorms were plastic allowing light to shine through. The sides of the dorms were netted to allow CO_2 to flow through freely. Every plant had its own pot to eliminate interaction through allelopathy or belowground VOCs. The dorms were stationed on shelves with five levels with three bug dorms on each shelf (Figure 2).

To test the effects of intraspecific competition on plant growth in a cultivar mixture, five of the boxes contained a mix of the cultivar, 'Salome' and 'Fairytale' (SF) with a 50:50 ratio. To test effects of intraspecific competition on the plant growth in pure stands, five boxes only contained the cultivar 'Fairytale' (FF) and five boxes only contained the cultivar 'Salome' (SS). This made a total of thirty bug dorms per trial with fifteen dorms for each CO_2-concentration. One box from each group was placed on each shelf intermittently (Table 2). The distribution was the same for both chambers. During one trial, ninety plants grew in each chamber making a total of 180 plants in both chambers. Since the same trial ran twice with slight moderations, the total number of plants was 360.

To get a more even growth, the seeds were germinated beforehand (Figure 3). For the first trial the seeds were put on wet paper in a plastic container for germination one day before sowing. For the second trial the seeds were put on wet paper for germination two days before sowing to minimize loss of data due to ungerminated seeds.

Figure 3. Germination of seeds.

2.3. Data collection

After two or three weeks depending on the trial duration, the plants were harvested for measuring. The stem was cut at the surface of the soil. The height of the longest leaf from each pot was measured in mm (Figure 4).

Figure 4. Measurement of height of the longest leaf.

In addition, the fresh weight of the plants was measured in gram (Figure 5). After the fresh weight and height had been measured the plant material were put in separate labelled paper bags and put in an oven of 80 °C for two days to be dried. An additional weighing of the dried material was then done in gram.

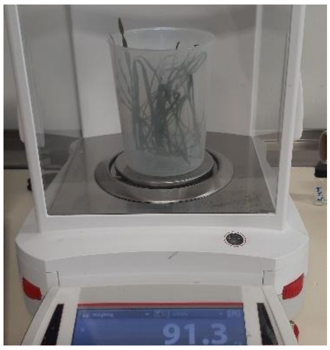

Figure 5. Measurement of dry weight.

2.4. Data analysis

Since there were many different groups with similar sample sizes, one-way ANOVA was used to test both two hypotheses. Separate one-way ANOVAs was conducted on the means of each examined parameter, height, fresh weight, and dry weight. When the p-value from a one-way ANOVA is smaller than the significance level of 0.05 it means that there is a statistically significant difference between the means of the groups. However, the one-way ANOVA doesn’t show where differences lie. To find out which groups that had statistically significant different means a Tukey Kramer procedure was run. Tukey Kramer was chosen because of unequal sample sizes. In the cases when the p-value of the one-way ANOVA was smaller than 0.05, then the one-way ANOVAs was followed by a Tukey Kramer procedure. All tests were conducted in excel and all graphs were made using excel. No test was performed to check for homogeneity of variance before running the ANOVAs.

3. Results

The results are divided into three sections. First, the effects regarding CO_2-concentrations will be displayed. The second section of the results zooms in on the two cultivars separately to get a closer look at how they responded to intraspecific competition when grown in pure stands compared to in a cultivar mixture. Lastly, in the third section of the results, variability of the dataset is addressed.

3.1. CO_2-concentration

Elevated levels of CO_2 increased the biomass of all groups in both the trial that ran for two weeks as well as the trial that ran for three weeks (Figure 6). Regarding fresh weight and dry weight there was a difference between the two CO_2-treatments ($p \leq .05$). A CO_2-concentration of 1000 ppm resulted in overall more biomass than 400 ppm. This was the case for both trial 1 and 2. However, regarding height, significantly different means were only found between some groups and treatments (Figure 6E & F).

In the first trial, there was a trend that intraspecific competition in a pure stand with only ‘Salome’ resulted in the most overall fresh weight, dry weight, and height in both 400 and 1000 ppm. There was also a trend that the second most biomass and height recorded occurred through intraspecific competition with a cultivar mix of ‘Salome’ and ‘Fairytale’. Lastly there was a trend that intraspecific competition in a pure stand with only ‘Fairytale’ resulted in the least amount of biomass and height.

In the second trial the trends differed. Now the results indicated that the cultivar mixture with ‘Salome’ and ‘Fairytale’ resulted in the most amount of fresh weight and dry weight under 400 ppm but the least in 1000 ppm. There was also a trend that intraspecific competition in a pure stand with only ‘Salome’ resulted in the most biomass in 1000 ppm. However, regarding height intraspecific competition in a pure stand with ‘Salome’ resulted in the shortest plants in both 400 and 1000 ppm whilst intraspecific competition in a pure stand with only ‘Fairytale’ resulted in the highest plants (Figure 6E and 6F).

Figure 6. Effect of CO_2-concentration on the plant growth of two cultivars of Barley

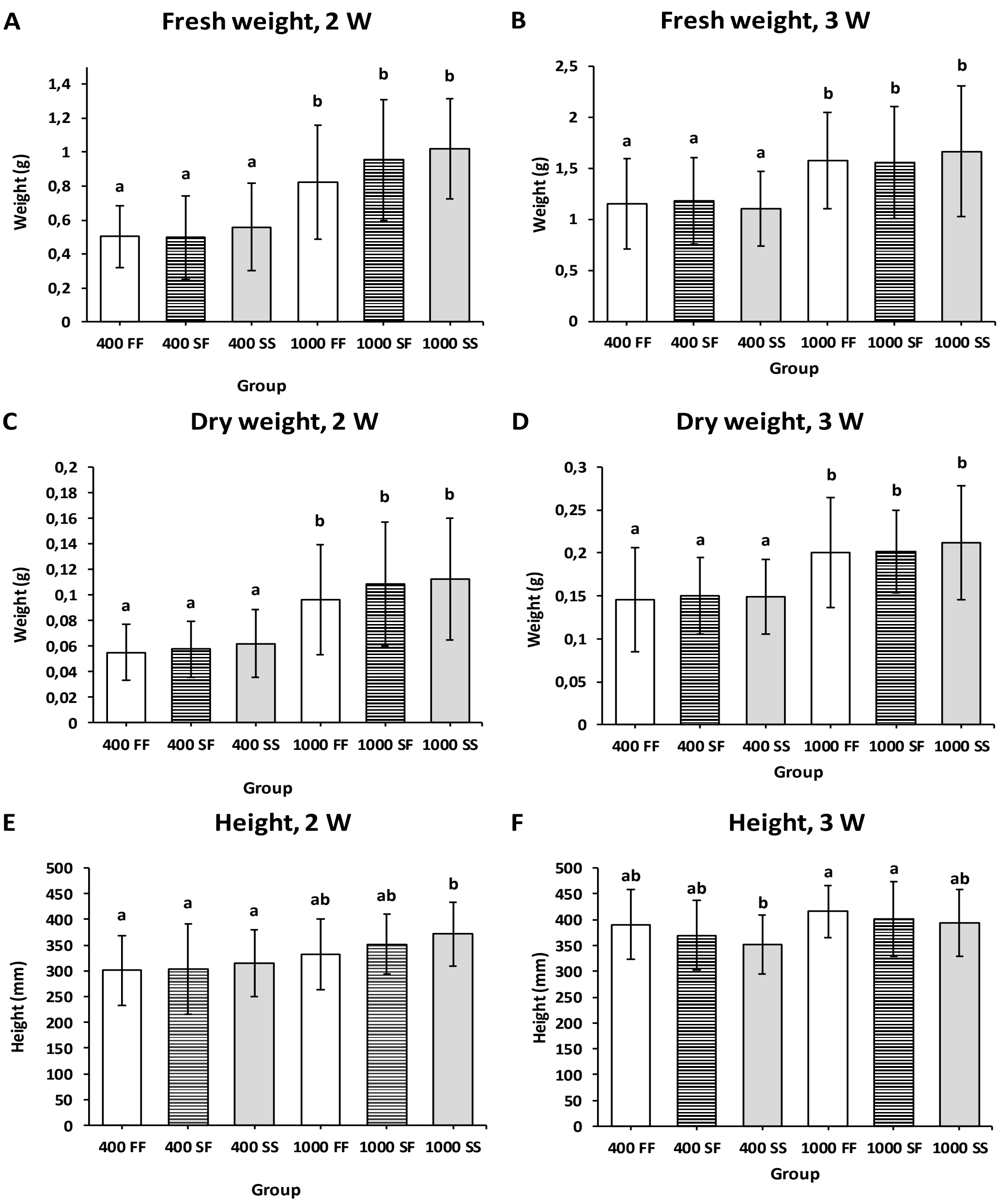

*Figure 6. Effect of CO_2-concentration, 400 and 1000 ppm, on the plant growth of two cultivars of Barley after two weeks (2 W) and three weeks (3 W). (**A & B**) Fresh weight, (**C & D**) dry weight, and (**E & F**) height. n-value = 25-30. FF signifies intraspecific competition in a pure stand with only 'Fairytale', SS signifies intraspecific competition in a pure stand with only 'Salome', SF signifies intraspecific competition in a cultivar mixture between 'Salome' and 'Fairytale'. Values are mean ± SD. Values are mean ± SD. Letters above bars represent statistical significance at $p \leq 0.05$. Statistical analysis by one-way ANOVA with Tukey Kramer procedure.*

After two weeks the biggest increase in dry weight between 400 and 1000 ppm occurred to the group with a cultivar mixture (Table 2). However, in the second trial of three weeks the group with a cultivar mixture had the least increase in biomass. Instead, the group with a pure stand of 'Salome' had the biggest increase in dry weight between the chambers with 400 ppm and 1000 ppm.

Regarding height intraspecific competition involving a pure stand of 'Salome' had the biggest increase in height in both trial 1 and trial 2. After two weeks the increase in biomass was 17,9 %. After three weeks the increase was 12,1 %. A pure stand with 'Fairytale' resulted in the lowest increase in height in both trials.

Table 2. The increase in dry weight and height between the 400 and 100 ppm of CO2.

Dry weight		
Trial	**Group**	**Increase (%)**
2 W	SS	*81,18*
	SF	*88,09*
	FF	*74,19*
3 W	SS	*42,08*
	SF	*34,38*
	FF	*37,99*
Height		
Trial	**Group**	**Increase (%)**
2 W	SS	*17,88*
	SF	*15,99*
	FF	*10,16*
3 W	SS	*12,05*
	SF	*8,43*
	FF	*6,51*

3.1. Cultivar mixture vs pure stand

3.1.1. The cultivar 'Fairytale'

For the cultivar 'Fairytale', no statistically significant difference was found between intraspecific competition in a cultivar mixture compared to intraspecific competition in a pure stand under neither of the CO_2 treatments after two and three weeks respectively (Figure 7). However, the data showed some indications.

In the first trial of two weeks, there was a trend that intraspecific competition with a cultivar mixture increased biomass and height for the cultivar 'Fairytale'. All parameters measured, fresh weight, dry weight and height increased slightly when 'Fairytale' was grown together with 'Salome' in both CO2-concentrations of 400 and 1000 ppm. The difference was somewhat bigger in the chamber with 1000 ppm.

In the second trial with a one-week extended growth period, the data showed the opposite tendency. After three weeks, intraspecific competition in a pure stand with only the cultivar 'Fairytale' resulted in more fresh weight, dry weight, and height, although marginally. Again, the difference was bigger in the chamber with 1000 ppm.

Figure 7. Effect of intraspecific competition on plant growth of the cultivar ‘Fairytale’

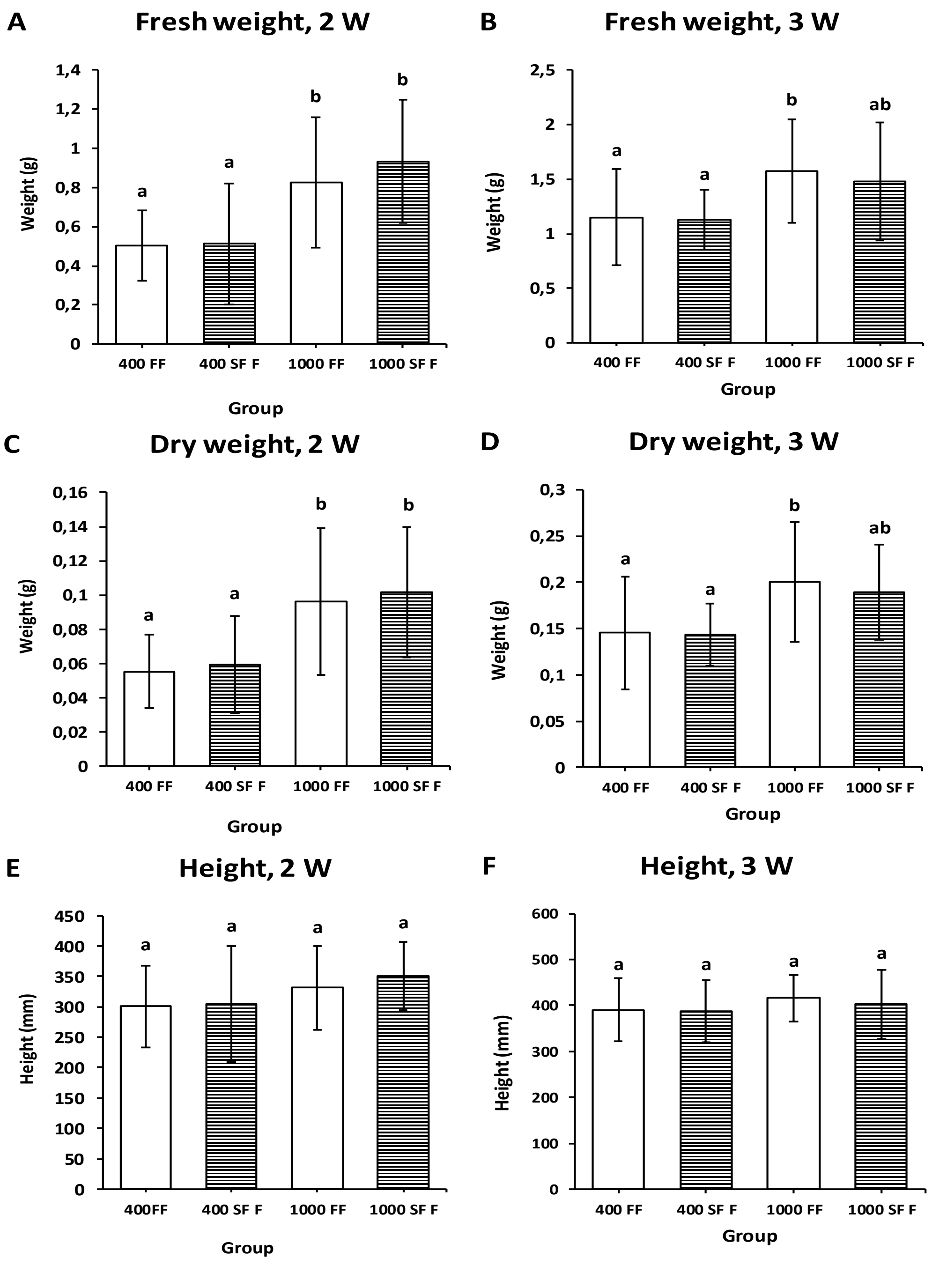

*Figure 7. Effects of intraspecific competition on the plant growth of the cultivar ‘Fairytale’. ‘Fairytale’ exposed to ‘Fairytale’ (FF), ‘Fairytale’ exposed to ‘Salome’ (SF F). Two treatments of CO_2-concentration, 400 ppm and 1000 ppm. (**A & B**) Fresh weight, (**C & D**) dry weight, and (**E & F**) height. n-value = 12-29. Values are mean ± SD. Letters above bars represent statistical significance at $p \leq 0.05$. Statistical analysis by one-way ANOVA with Tukey Kramer procedure. 2 W = two weeks, 3 W = three weeks.*

3.1.2. The cultivar ‘Salome’

For the cultivar ‘Salome’, no statistically significant difference was found between intraspecific competition involving a cultivar mixture compared to intraspecific competition in a pure stand under neither of the CO_2 treatments after two and three weeks respectively (Figure 8). However, like with the cultivar ‘Fairytale’, the data showed some indications worth noting.

In the first trial of two weeks there was a trend that the plant growth of the cultivar ‘Salome’ was affected negatively when it grew intraspecifically in a cultivar mixture together with ‘Fairytale’. However, with one exception, see group 1000 SF S (Figure 8C). The cultivar ‘Salome’ had a slight increase in dry weight when grown in a cultivar mixture together with ‘Fairytale’ in the chamber with a CO_2-concentration of 1000 ppm.

In the second trial of three weeks the trend was opposite. Then, the cultivar ‘Salome’ benefitted slightly from intraspecific competition in a cultivar mixture with ‘Fairytale’ under both CO_2-treatments with one exception, see group 1000 SS (Figure 8B). The fresh weight was slightly bigger in the pure stand with only ‘Salome’ in the chamber with 1000 ppm.

Figure 8. Effects of intraspecific competition on the plant growth of the cultivar ‘Salome’

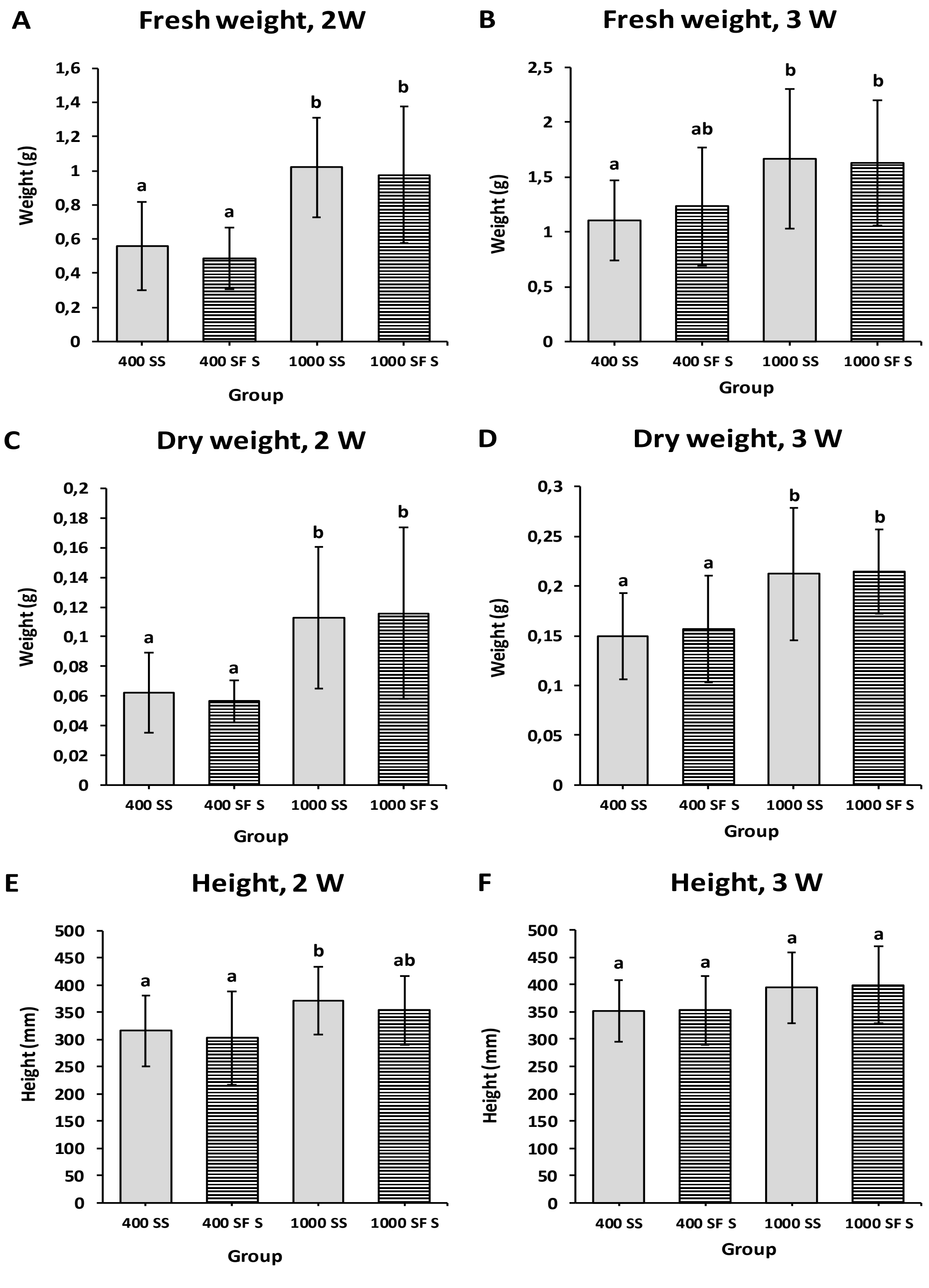

*Figure 8. Effects of intraspecific competition on the plant growth of the cultivar ‘Salome’. ‘Salome’ exposed to ‘Salome’ (SS), ‘Salome’ exposed to ‘Fairytale’ (SF S). Two treatments of CO_2-concentration, 400 ppm and 1000 ppm. (**A & B**) Fresh weight, (**C & D**) dry weight, and (**E & F**) height. n-value = 12-29. Values are mean ± SD. Letters above bars represent statistical significance at $p \leq 0.05$. Statistical analysis by one-way ANOVA with Tukey Kramer procedure. 2 W = two weeks, 3 W = three weeks.*

3.2. Variation

There was a big standard deviation in the data. To investigate why, the different means of each group from every shelf was compared to see if distance from the light source caused the variation. The height and dry weight from both the first and the second trial were combined to investigate the overall trend. The upper most shelf is shelf number one, and the bottom shelf is shelf number five.

3.2.1. Height

Plants located on lower shelves were higher on average compared to plants on the upper most shelf (Figure 9).

Figure 9. Light variation, Height

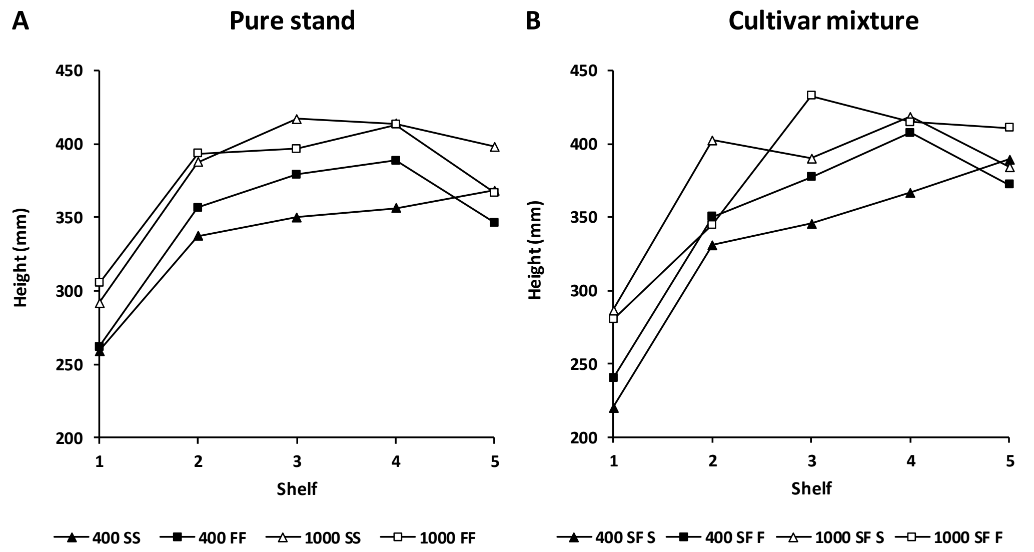

Figure 9. Mean height of plants grown on different shelfs showing variation in light exposure. SS = 'Salome' exposed to 'Salome', FF = 'Fairytale' exposed to 'Fairytale', SF S = Salome exposed to 'Fairytale', SF F = 'Fairytale' exposed to 'Salome'. CO_2-level, 400 and 1000 ppm. ***(A)*** *Intraspecific competition in pure stand.* ***(B)*** *Intraspecific competition in cultivar mixture. n-value = 6-12.*

3.2.2. Dry weight

When looking at the means of dry weight there was another trend. Overall, most dry weight was found on the middle and upper shelves and the dry weight decreased towards the lower shelves (Figure 10). However, one group diverged from the trend. It was the groups with intraspecific competition involving a cultivar mixture of 'Salome' and 'Fairytale' on shelf 3 in the chamber with a CO_2-concentration of 400 ppm (Figure 10B).

Figure 10. Light variation, Dry weight

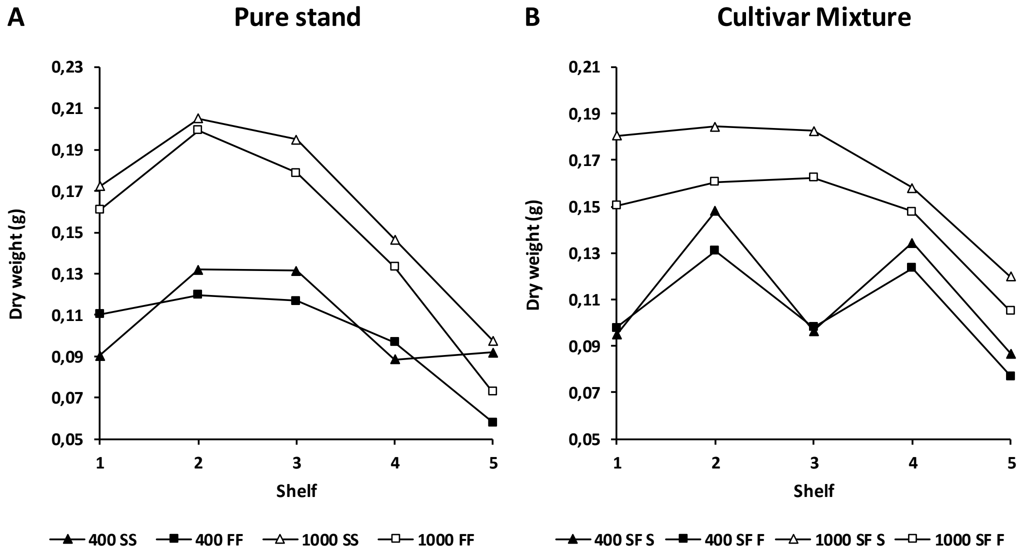

*Figure 10. Mean dry weight of plants grown on different shelves showing variation in light exposure. SS = 'Salome' exposed to 'Salome', FF = 'Fairytale' exposed to 'Fairytale', SF S = Salome exposed to 'Fairytale', SF F = 'Fairytale' exposed to 'Salome'. CO_2-level, 400 and 1000 ppm. (**A**) Intraspecific competition in pure stand. (**B**) Intraspecific competition in cultivar mixture. n-value = 6-12.*

4. Discussion

The first research question was: does intraspecific competition involving a cultivar mixture lead to more plant growth compared to intraspecific competition in a pure stand? In contrast to previous studies (Essah & Stoskopf 2002; Zhang & Wang 2011) no difference was found between the group involving a cultivar mixture and the groups involving pure stands ($p \leq .05$). Even so, the results showed some tendencies that sometimes aligned with, and sometimes diverged from previous findings. In the first trial of two weeks, there was a trend that the cultivar ‘Fairytale’ had an increased plant growth when growing in a cultivar mixture, whilst ‘Salome’ when growing in a pure stand. However, this was not the case for one of the groups. In the second trial with a one-week extended growth period, the data showed the opposite tendency. After three weeks, the cultivar ‘Fairytale’ instead showed a tendency of increased plant growth when growing in a pure stand, whereas ‘Salome’ when growing in a cultivar mixture. In the cases where a cultivar mixture promoted plant growth the tendencies aligned with previous finds (Essah & Stoskopf 2002; Zhang & Wang 2011). However, the results also showed the opposite tendency contradicting previous findings.

Information regarding the nutritional requirements of the two cultivars of Barley included in the trial haven’t been found. Assuming they have similar resource demands, it is possible that ‘Salome’ and ‘Fairytale’ have developed unique strategies to compete since there was indications that they exhibited different growth rates. In the first trial that ran for two weeks ‘Salome’ fared better then ‘Fairytale’ regarding overall biomass and height. However, in the second trial, which had a one-week extended growth period, ‘Fairytale’ caught up with ‘Salome’ and the difference between them was not as apparent anymore. This indicates that ‘Fairytale’ needs a bit more time to establish itself. Whilst ‘Salome’ focused on elongation and on allocating resources to aboveground plant tissue in early stages, similar to some barley cultivars (Kegge et al., 2015), it could be that ‘Fairytale’ was allocating resources to establish a more extensive root system as has been seen in other barley cultivars (Ninkovic 2003). To test if this is the case future studies should include measurements of root growth.

Usually, growers want to limit competition as much as possible whilst at the same time utilizing the available space to the fullest by growing crops at a high density (Brosset & Blande 2022). Increased yields in pure stands have been a priority for plant breeders (Bourke et al. 2021). As a result, they have produced cultivars that are very productive and specialized. However, the specific traits of the cultivars are not always well adapted to systems that use intercropping, since they haven't been bred for that purpose (Reusch et al. 2005; Bourke et al. 2021). One explanation to why the results didn't fully align with previous studies could be that two cultivars in this trial have been bred to be more adapted to growing by themselves in pure stands. Other possibilities could be that the sample size was too small, or that the duration of the trial should have been increased to cover more of the growth cycle of the plants.

Another possibility could be that the arrangement with bug dorms was not an appropriate method to use. Since the bug dorms were not airtight, the plants have probably been exposed to more volatiles then intended. Furthermore, there were other experiments occurring in the same chamber during the period of the trial. When examining the distance of how far volatile compounds move from the emitter plant it was found that after 50 cm, volatiles that induce resistance in lima beans had a reduced impact on the receiver (Heil & Adame-Álvarez 2010). This suggests that effects of volatile compounds are stronger on plants in closer proximity to the emitter. However, all chemical compounds have unique characteristics which likely impacts their dispersion within the chambers. Furthermore, factors such as temperature, humidity and draft would most probably impact how far different chemicals can move. To make the results more reliable future trials could increase the distance between the bug dorms or keep the different groups in separate chambers all together.

A factor that likely affected the results was that the standard deviation of the dataset was large. It was most probably a result of differences in light-availability. Plants located on lower shelves were higher on average compared to plants further up. When looking at the means of dry weight there was another trend. Overall, most dry weight was found on the middle and upper shelves and the dry weight decreased towards lower shelves. Plants on lower shelves got more shaded, probably resulting in elongation responses (Craine & Dybzinski 2013), whilst plants further up could focus more on increasing their total leaf area and overall biomass. When the data set is so spread out it is harder to find statistical differences since the values are not grouped together. To get a more even dataset, in future trials, all dorms should be put on the same level. This would factor out variations caused by differences in light-availability and the standard deviation of the data set would most likely become smaller, possibly resulting in more clear results. Another aspect to consider is that when plants are facing multiple stresses, they tend to focus on one of the stresses (Lamers et al. 2020). It is possible that the stress of not receiving enough light was prioritized by plants on lower shelves instead of them focusing on the competition present.

Another factor that reinforces the point that future trials should keep all plants on the same level is the properties of CO_2. CO_2 is a denser compound then air. It is possible that it sank towards the floor of the chambers making the concentration of CO_2 larger for plants on lower shelves. However, in a greenhouse trial with strawberry, the concentration of CO_2 was larger closer to the ceiling due to convection (Zhang et al. 2020). As warm air rises whilst cold are sinks, the CO_2 can follow the warmer air. This could result in a larger concentration for plants located on shelves further up in this trial. All the lamps were stationed in the ceiling, so it is possible that it was warmer further up compared to further down in the chambers.

Even though a cultivar mixture didn't promote plant growth more than the pure stands, it should still be a factor to keep in mind when breeding cultivars for future conditions since it has been shown to be beneficial in previous studies (Reusch et al. 2005; Zippinger-Dingley et al. 2014). Higher diversity in a population makes the population more resilient (Lin 2011). Since there are more traits and a bigger genetic variation the chances of handling biotic and abiotic stresses increase (Lin 2011). In a world with changing global climate conditions, it is risky to be too dependent on monocultures. Whilst it is efficient and less costly it comes with other downsides. For example, barley has been shown to negatively impact the quality of soil in terms of nitrogen and carbon content when grown in a pure stand (Chapagain & Riseman 2014). Plant breeders should strive to breed crops that thrive in systems that use intercropping to make the population more stable when faced with future challenges arising from changed climatic conditions. Especially considering that the cultivars that exists today experienced challenges when faced with predicted climatic conditions (Ingvordsen et al. 2015).

The second research question was: Do elevated levels of CO_2 effect the intraspecific competition of barley? Since there were no statistically significant effects of the two forms of intraspecific competition, no conclusions can be made regarding the impacts of elevated levels of CO_2 on intraspecific competition. In the first trial with dry weight, intraspecific competition in a cultivar mixture resulted in the biggest increase in plant growth between 400 and 1000 ppm. However, in the second trial intraspecific competition in a pure stand with 'Salome' resulted in the biggest increase in plant growth between the two CO_2-concentration. Regarding height, the biggest increase between 400 ppm and 1000 ppm occurred in a pure stand with 'Salome' whilst the least increase occurred in a pure stand with only 'Fairytale'.

This was the case for both the first and the second trial. It is possible that this is an indication that elevated levels of CO_2 effects the competitive ability of cultivars differently, similarly to when soybean grew together with different weeds (Miri et al. 2012). However, without further research it is simply speculations. To fully understand how plant-plant interactions are affected by elevated levels of CO_2, more extensive studies examining a wider range of different cultivars should be conducted.

One thing that is certain is that elevated levels of CO_2 affected plant growth positively which is in accordance with previous studies (Taub 2010; Gardi et al. 2022). Difference was found between the two CO2-treatments concerning dry weight and fresh weight ($p < .05$). Overall, in the chamber with 1000 ppm the plants became bigger and had more biomass. Regarding height, difference was only found between some of the groups. Since raised levels of CO_2 had positive effects on the plant growth it can prove to work as a resource in a future where agriculture will face big challenges (Ingvordsen et al. 2015). One thing to note though is that the trial was taken place inside in chambers with controlled conditions. It is not certain that the plants would react in the same way outside in an open field setting were there are other forces at play. Outside, plants are faced with numerous factors which makes things more complex (Suzuki et al. 2014; Lamers et al. 2020). Nonetheless, plant breeders have to be prepared so they can breed crops that have an even higher responsiveness to future elevated levels of CO_2 (Mitterbauer et al. 2017).

4.1. Conclusions

The results are not sufficient to draw conclusions regarding how the two cultivars are affected by the different intraspecific interactions. However, based on earlier research and the tendencies that was seen in this trial, the cultivar in question should be considered when deciding which cropping system to use. Furthermore, plant breeders should strive to breed crops that thrive in systems that use intercropping to make the population more stable when faced with future challenges.

Whilst elevated levels of CO_2 have positive effects on the plant growth of barley more research is needed to determine how elevated levels of CO_2 effect the competitive ability of different cultivars. The interactions between neighbouring plants are complex and intertwined with a range of factors which should be considered when studying the responses. Future trials should focus on lowering the standard deviation by keeping all the plants at the same distance from the light source. To get more comprehensive results, the belowground interactions through allelopathy and belowground VOCs should also be included. A wider range of different barley cultivars should also be included.

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