Investigating the Physiological Effects of Fungicides on Sugar Beet Growth and Yield

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2nd year review

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Abstract

Sugar beet (*Beta vulgaris* subsp. *vulgaris*), commercially grown for its production of sugar, is susceptible to a range of pests and diseases. These stresses can result in significant yield loss, so there are a range of treatments available to combat disease, some of which have been observed to improve sugar yields even in cases of low disease pressure, suggesting activity aside from disease control contributing to improved productivity. Similar relationships have been observed in other crops, with varying mechanisms contributing to improved yields including a stay-green effect, improved stress tolerance, and more effective use of fertiliser. This project aims to uncover the mechanisms for these improved yields in sugar beet. Last year, the focus was on two separate experiments; a field trial with a large range of treatments, and a polytunnel trial comparing how plants under different treatments responded to drought stress. During the first year of trials, fungicide treatments including azoles, SDHIs, and azole + strobilurin combinations were higher than other treatments for physiological processes like CO_2 assimilation and stomatal conductance, and reflectance indices showed that these treatments also had higher chlorophyll content. In terms of harvest data, the top yielding treatments were the azole + strobilurin combination > the strobilurin > the SDHI, and further experiments could help to determine the specific physiological reasons for their success. Harvest data provided some potential insights, such as the azole + strobilurin combination and the strobilurin treatments yielding highest, and also both having the highest specific leaf area (leaf thickness). This year a combination of physiological data collection as well as genetic and enzymatic lab work will be carried out to investigate further into the effects of the fungicides on growth and yield. Studies in other crops have suggested that interactions with the ATP-synthesis pathway as a result of fungicide application can lead to altered enzyme activity in the plants, and that these interactions in turn can lead to better performance of the crops. This year, the aim is to determine some of these interactions in sugar beet, to gain a better understanding of the specific interactions leading to increased growth and yields under fungicide use.

Contents

1. Introduction

For a full introduction of the crop and its optimal growing conditions, please refer to my 1st year report. The introduction of this report will focus mainly on the concepts relevant to this year's experiments.

1.1 Nitrogen uptake

Uptake of nitrogen is important for the growth and canopy expansion of sugar beet, increasing the speed of canopy expansion and overall size of the final canopy. The optimal sugar beet leaf area index (LAI) is c.3, which will intercept 95% of incoming radiation. To achieve this, the crop needs to take up approximately 90-120kg N/ha. However, if plants are supplied with excessive nitrogen later in the season e.g. through mineralisation of organic soils or manure, the partitioning of biomass can be affected, leading to more biomass production in the leaves and less in the root. Another impact of excessive nitrogen usage is that it can cause an increase in free amino nitrogen in the root, an impurity which reduces the extractability of sugar.

1.2 Fungicides and their activity

Various types of fungicides can be applied to crops as a means of decreasing the incidence of disease and subsequent loss of yields. Fungicide's abilities are limited to protecting uninfected plants and halting the spread from infected plants, but unlike fungal treatments for animals, plant fungicides rarely have 'curative' properties, which are limited even if such properties are present.

Fungicides can be contact, translaminar or systemic. Contact fungicides protect only the areas of the plants which have been sprayed, but do not penetrate or relocate into the unsprayed sections. Translaminar fungicides redistribute the treatment from the sprayed surface of the leaf to the unsprayed surface, but do not extend throughout the plant. Systemic fungicides are taken up by the plant after application and are then redistributed via the xylem vessels through the plant.

There are three major types of fungicides; azoles, strobilurins (aka Qo inhibitors, or QoI), and succinate dehydrogenase inhibitors (SDHIs). For the main fungal diseases of UK sugar beet - powdery mildew and rust - most of the fungicides recommended by BBRO are azoles or combinations of azoles and strobilurins. For example, Escolta can be used to treat powdery mildew and rust, and the active ingredients are cyproconazole (an azole) and trifloxystrobin (a strobilurin).

1.2.1 Azoles

Azoles are a major class of systemic fungicides used for crops and are often used in combination with strobilurins when treating a range of foliar diseases. They act by suppressing ergosterol synthesis via the inhibition of 14 α-demethylase, which results in fungal cell growth inhibition. They are synthetic in origin and have a cyclic structure. Some key examples of azoles include imidazoles and triazoles.

1.2.2 Strobilurins

Strobilurins used on crops have been developed synthetically based on naturally occurring antifungal products found in the fungus *Strobilurus tenacellus*, with optimisation focusing on photostability and activity. They are mostly locally systemic/translaminar fungicides, being absorbed into the leaf cuticle but not being incorporated into the plant system. Some strobilurins are more mobile, for example azoxystrobin which moves translaminarly as well as systemically. The active process of strobilurins is to inhibit electron transfer in the mitochondria of target fungi, specifically targeting and disrupting Complex III, in order to halt metabolism. As the strobilurin group of fungicides are synthetic and similar in structure to one another, they can be prone to developing resistance. For this reason, it is highly important to use strobilurins in combination with another product from a different fungicide group.

1.2.3 Succinate Dehydrogenase Inhibitors (SDHIs)

SDHIs inhibit respiration in fungi by targeting complex II of the mitochondria, specifically blocking the ubiquinone-binding site (Q-site). Intensive use of this group of fungicides likely leads to higher selection pressures, ultimately contributing to increased resistance by fungal diseases. One effective way to reduce the risk of resistance is to use a combination of types of fungicides at once. Many fungicide products applied to sugar beet crop contain more than one type of fungicide.

1.3 Yield improvements

The increased use of fungicides on sugar beet crops in the UK in recent years has led to increases in yield of up to 20% (Stevens and Burks, 2012). While much of the improved yield is due to disease control, some of the improvements are attributed to physiological interactions within the plant (Ober et al., 2004). Ober et al. (2004) suggested that improved sugar beet yield in response to fungicides was not exclusively due to disease control, and that interactions were taking place to improve canopy persistence and efficiency. Studies on other crops have also shown similar relationships, including potatoes (MacDonald et al., 2007) and wheat (Ishikawa et al., 2012). Fungicide application could also improve yield by enhancing uptake and metabolism of nitrogen via increased activity of the nitrate reductase enzyme, a relationship which has been observed in other crops (Jabs et al., 2004).

Several studies have shown that fungicide use on various crops can lead to a ‘stay-green’ effect, where the area of green, photosynthetically active leaves is higher for longer (Sulewska et al., 2019). In sugar beet, this stay-green effect would allow for a longer period of sugar production, potentially leading to increased yield. Where studies have separated the fungicide into the active ingredients (i.e. investigating the strobilurin and azole separately), the stay green effect tends to be more apparent with the strobilurin (Bertelsen et al., 2001). This relationship has not yet been determined in sugar beet.

A possibility to consider when observing increased growth and yield in seemingly healthy crops treated with fungicides is that the response to fungicide may be partly due to the control of pre-symptomatic disease. An example of this situation occurring could be that disease is recorded in one small area of a field and the entire field is treated; the plants which looked uninfected may have already been infected with the disease, but had not yet shown symptoms.

Last year’s trials aimed to reveal how treatment with different fungicide groups affected plant physiology and resulting yields, with weekly physiological readings followed by final harvest data. There was a focus on stress tolerance in the polytunnel trial, while the field trial focused on a larger range of fungicide treatments and a comparison between two varieties of differing disease susceptibility. The smaller trial gave some interesting indications of treatment interactions with plant physiology, but most of the highly significant data came from the larger field trial. These first year experiments (2021) are summarised in the first section of this report, with a focus on this year’s experiments (2022) later in the report.

1.4 Overview of year 1 experiments

Aims

i. **Polytunnel:** To investigate the effects of drought stress on sugar beet while treated with an SDHI, a strobilurin or left untreated.

ii. **Field:** To investigate the effects of a large range of fungicides (including fungicide group combinations) on sugar beet physiology and resulting yields.

Objectives

i. **Polytunnel:** Compare plants treated with either an **SDHI**, a **strobilurin**, or left untreated under both well-watered and droughted conditions.
    a. Compare physiological readings such as canopy greenness, chlorophyll fluorescence, and a range canopy reflectance indices.
    b. Compare biomass production and sugar yields.

ii. **Field:** Compare plants treated with either an **azole**, an **SDHI**, an **azole + SDHI combination**, an **azole + strobilurin combination**, a **strobilurin**, or left untreated.
    a. Compare physiological readings such as canopy cover %, canopy greenness and a range of canopy reflectance indices.
    b. Compare biomass production and sugar yields.
    c. Compare disease presence and persistence.

Chapter A - Polytunnel experiment 2021

2. Methods

2.1 Experimental design

Variety

Kortessa (KWS) – low susceptibility to both powdery mildew and rust compared to others on the BBRO recommended list.

Treatments

1. No treatment
2. SDHI
3. Strobilurin

Plants were treated three times, with the same fungicides, on 11 June, 5 July, and 2 August 2021.

2.2 Data collection

2.2.1 SPAD

A SPAD chlorophyll meter (SPAD, Minolta Camera Co., Osaka, Japan) was used approximately weekly in the polytunnel from 11 June to 22 September to record leaf greenness. The two newest fully emerged leaves were used from each of the two central sample plants, for a total of four readings per box. As the SPAD meter is known to produce highly variable values, three readings were taken per leaf and the mean was used as the value for that leaf.

2.2.2 CO_2 assimilation and stomatal conductance

An LI-6800 portable photosynthesis system (LICOR, Lincoln, Nebraska, USA) was used on 29 September 2021 to compare CO_2 assimilation and stomatal conductance between treatments. The newest fully emerged leaf was chosen, and the leaf clip was closed onto the leaf until all conditions within the clip were showing as stable on the LICOR. The measurement was taken and later exported from the LICOR for processing.

2.2.3 Harvest data

Plants were harvested on the 5 October 2021. From each box, the central two plants were used for further analysis in the lab (top weight, root weight, leaf area etc.), while the outer 10 plants were topped, and the roots sent to BBRO for sugar % and impurity analysis.

2.3 Data analysis

An analysis of variance (ANOVA) suitable for the split-plot design was carried out using Genstat 19th edition (VSN International, Hemel Hempstead, UK), using fungicide treatment and irrigation as factors, and including blocking in the analysis. Where significant differences were reported in the ANOVA, multiple comparisons were calculated using Duncan's multiple range tests, with a confidence interval of 95%. Where data were collected repeatedly over several weeks, a repeated measures ANOVA was used. Graphs were developed in Microsoft Excel, using values of the least significant differences as error bars.

3. Polytunnel results

3.1 SPAD

SPAD was highly variable throughout the season, and even under a repeated measures analysis, there was no significant interaction between fungicide treatment and irrigation, though a difference can be seen when plotted on a graph. Under well-watered conditions, the greenness was higher when treated with the SDHI or left untreated, compared with the strobilurin. However, under droughted conditions, the strobilurin treatment was associated with a higher greenness than the SDHI and untreated control. For ease of understanding and interpretation, the graphs showing well-watered and droughted conditions have been included separately, below in **Figures 1** and **2**.

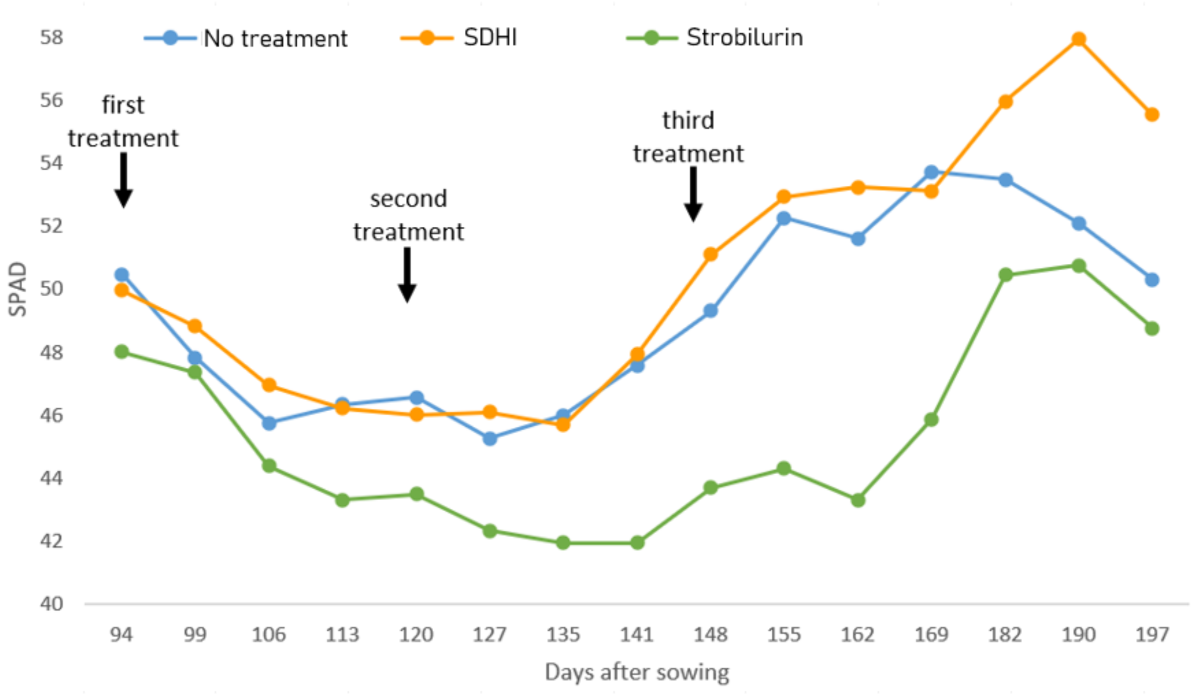

Figure 1. Leaf greenness (SPAD) depending on treatment with either an SDHI, strobilurin, or non-treated control under well-watered conditions.

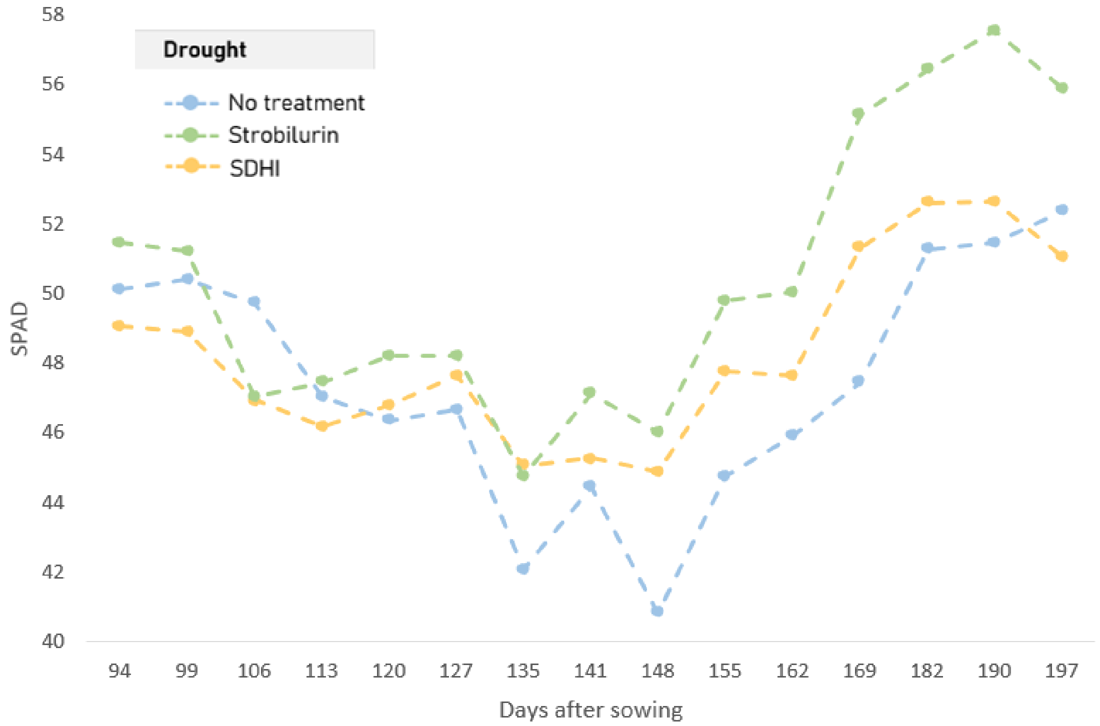

Figure 2. Leaf greenness (SPAD) depending on treatment with either an SDHI, strobilurin or non-treated control, for plants growing in droughted conditions.

These results suggest that the strobilurin might help the plant to stay greener even while undergoing drought stress, however this interpretation could be misleading, due to the tendency for SPAD to have higher readings when leaves are extremely drought stressed, as the leaves become thinner and chlorophyll becomes more concentrated. Chlorophyll extraction would help to determine the true nature of this relationship.

3.2 CO_2 assimilation and stomatal conductance

In general, the plants performed similarly regardless of treatment when droughted, but under well-watered conditions, the SDHI and strobilurin treatments had higher CO2 assimilation (P=0.016, **Figure 3**) and stomatal conductance (P=0.029, **Figure 4**) than the control.

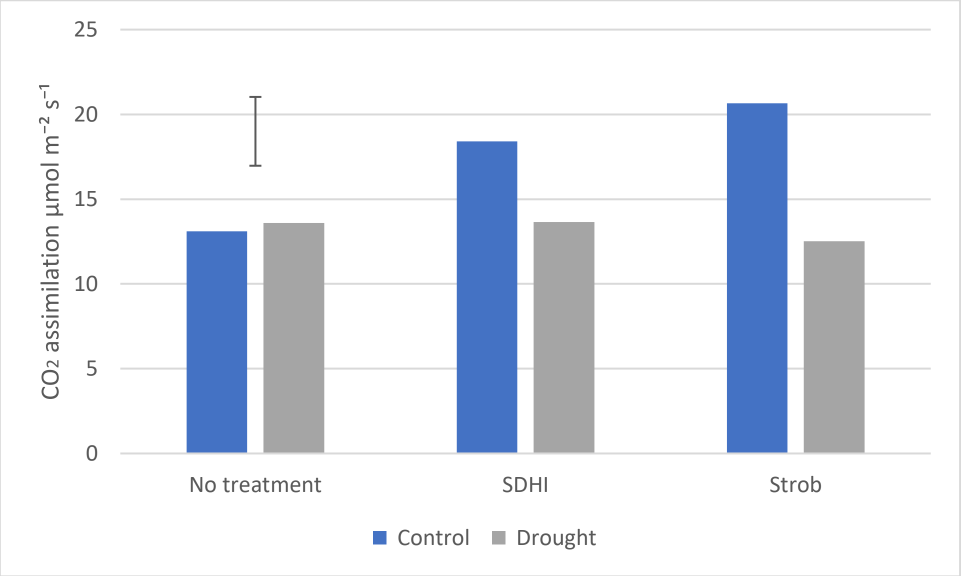

Figure 3. Comparison of CO_2 assimilation between treatments depending on watering regime.

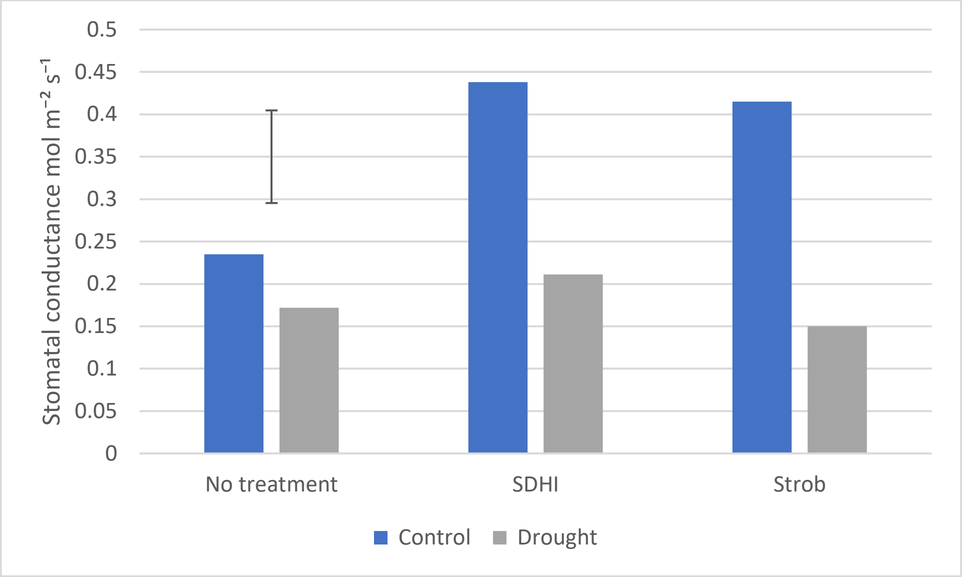

Figure 4. Comparison of stomatal conductance between treatments depending on watering regime.

3.3 Harvest data

Fungicide treatment had no effect on leaf area of droughted plants, however under well-watered conditions, SDHI and strobilurin treated plants had much larger leaf area than the control (P=0.04, **Figure 5**).

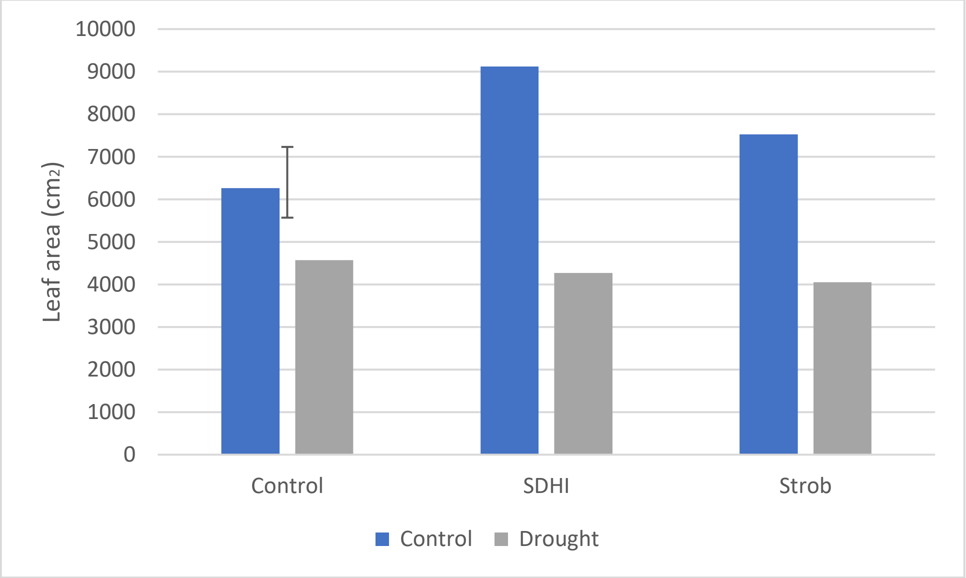

Figure 5. Comparison of total leaf area depending on treatment with either an SDHI, strobilurin or untreated control when undergoing either well-watered or droughted conditions.

The dry weight of roots from plants treated with the SDHI were significantly larger than the roots from plants treated with the strobilurin (P=0.045, **Figure 6**)

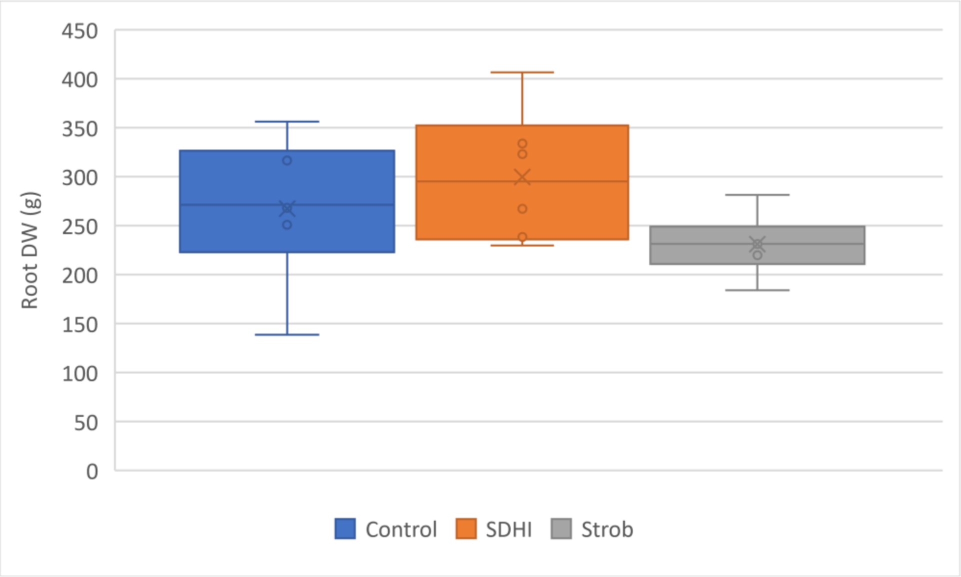

Figure 6. Comparison of root dry weight between plants treated with an SDHI, a strobilurin or left untreated.

When the total sugar yield was calculated using the clean weights of the outer 10 plants of each box and the sugar % returned from the processing factory, there were no significant differences between fungicide treatments. The order of the treatments from highest to lowest was still the same as the dry weights in **Figure 6**; SDHI > control > strobilurin, but these differences were not significant. The edge effects in the boxes were severe, so large variation may have been the cause of this.

Chapter B - Field experiment 2021

4. Methods

4.1 Experimental design

Varieties

Two varieties were chosen for the field trial, with the purpose of providing some insight into the extent to which disease control (or even pre-symptomatic disease control) is responsible for the increase in yields. If the two varieties with differing disease susceptibility responded differently from each other, it would indicate a larger proportion of improved yields being due to early disease control. The two varieties used in this trial were:

- Kortessa (KWS), low susceptibility to foliar diseases

- Advena (KWS), higher susceptibility to foliar diseases

Treatments

0. No treatment
1. Azole
2. SDHI
3. Azole + SDHI
4. Azole + Strobilurin
5. Strobilurin

The field was treated twice, with the same fungicides, on the 3 August and the 7 September 2021.

4.2 Data collection

4.2.1 SPAD

The SPAD chlorophyll meter was used under the same protocol as in the polytunnel experiment. Four plants from within the central rows of each plot were randomly selected, and the newest fully emerged leaf was measured.

4.2.2 Reflectance indices

The ASD Fieldspec was used under the same protocol as in the polytunnel experiment. Four plants from within the central rows of each plot were randomly selected, and the newest fully emerged leaf was used.

4.2.3 Photography

Photographs were taken of the plots from above using a Canon DLSR camera attached to a tractor driving alongside the plots. Each plot was photographed from two sides, which provided images covering 72% of the plot area. Photographs were analysed for canopy cover % using ImageJ software (NIH, Bethesda, Maryland, USA), using a colour thresholding area analysis (thresholds at: Hue 33 187, Saturation 0 255, Brightness 0 255).

4.2.4 Crop Circle

Further reflectance data was collected using a Crop Circle canopy sensor kit (Holland Scientific, Lincoln, Nebraska, USA) attached to a tractor driving alongside the plots.

4.2.5 Disease scoring

Disease scoring was recorded during the week commencing 15th November 2021, to quantify the presence of common diseases such as rust and cercospora leaf spot.

4.2.6 Harvest processing

Plots were initially harvested in December 2021 by removing 10 plants from each plot by hand for lab processing. The remaining plants in each plot were then removed using a plot harvester before being sent for analysis at the BBRO tarehouse at Wissington beet sugar factory. The hand-harvested plants were used for measurements which included the tops of the plants and dry weights, whereas the machinery-harvested plants were used in analysis of measurements such as sugar % and impurity content.

In the field, the roots and tops were weighed separately immediately after harvest. In the lab, more intricate measurements were taken such as the clean weight of roots, petiole weight, leaf weight, and dead leaf weight. Additionally, a leaf area meter was used to measure the area of a sub-sample of the leaves. Measurements were combined to find further information such as specific leaf area (calculated as grams per cm^2 area of leaf, as an indication of the thickness of leaves).

4.3 Data analysis

Data analysis was carried out as described in section **2.3** in the polytunnel section of this report.

5. Field results

5.1 SPAD

A repeated measures ANOVA revealed that the azole + strobilurin, azole + SDHI, and SDHI treatments consistently had higher SPAD values than the other treatments (P=0.001, **Figure 7**).

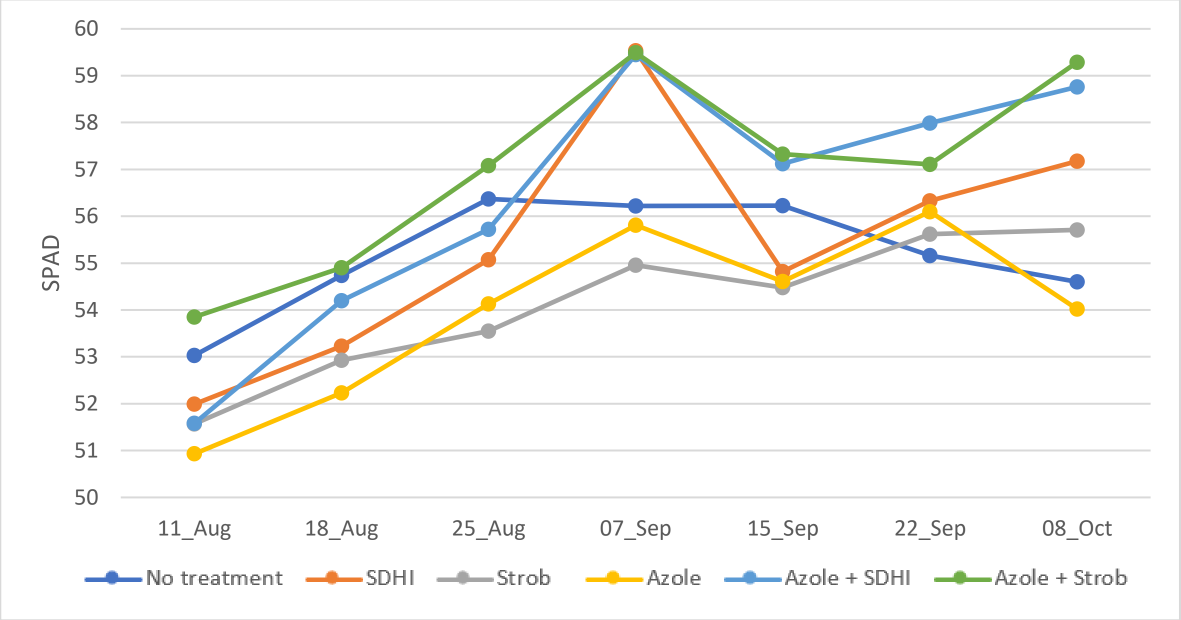

Figure 7. The effect of fungicide on SPAD readings throughout the season.

5.2 Reflectance indices

5.2.1 Triangular vegetation index

Using a repeated measures analysis, the strobilurin had the highest TVI value, with the azole + strobilurin combination, and non-treatment control having the lowest values (P=0.009, **Figure 8**). This index is positively associated with chlorophyll content.

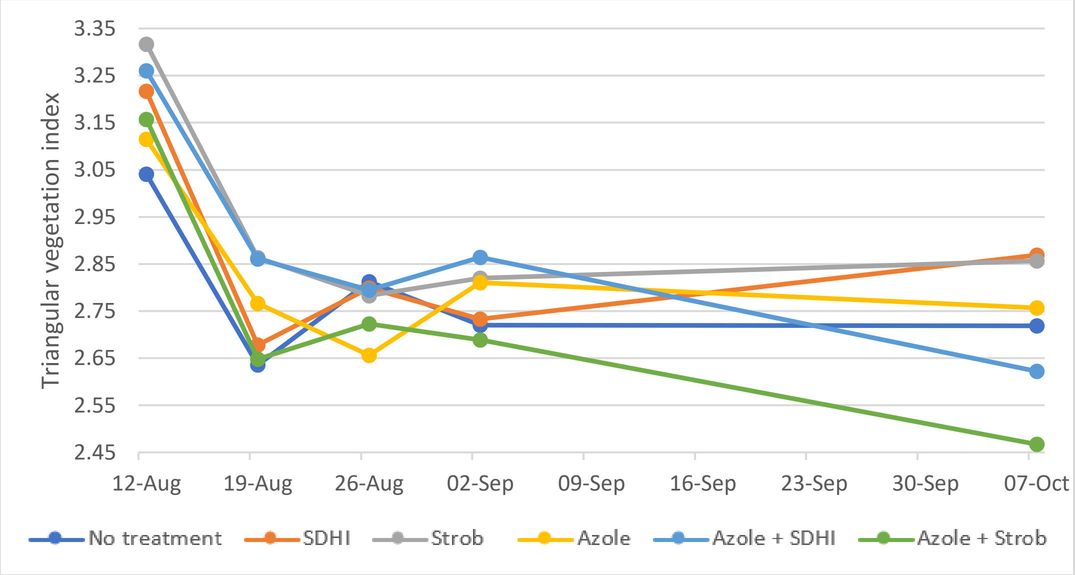

Figure 8. Comparison of the effects of fungicides on triangular vegetation index over time.

5.2.2 RARSb

Using a repeated measures analysis, the highest RARSb values were from the azole + strobilurin combination, and the untreated control (P=0.021, **Figure 9**). This index gives an indication of the chlorophyll b content in leaves, and is positively associated with biomass production.

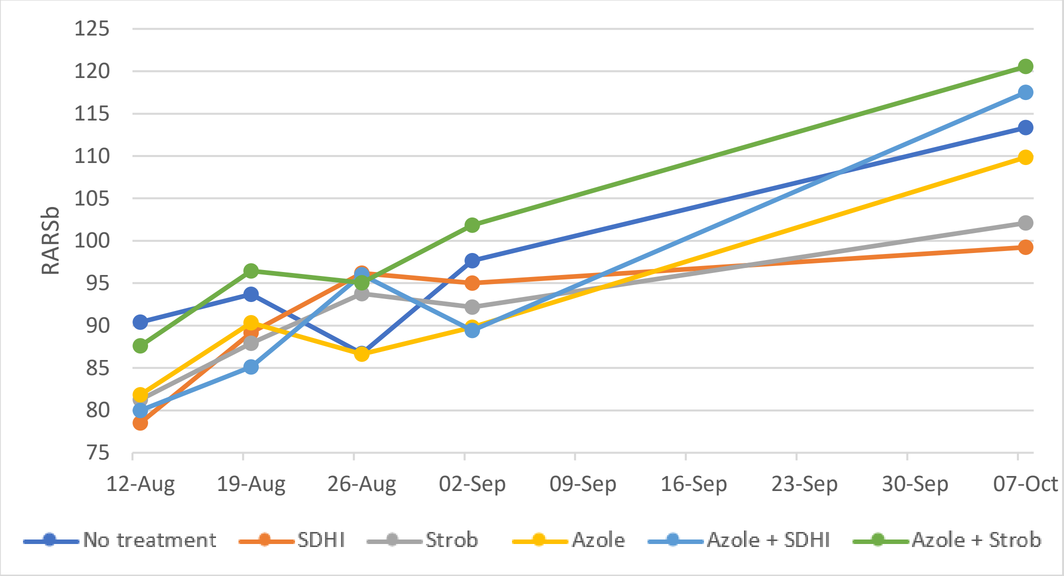

Figure 9. Comparison of the effects of fungicides on RARSb over time.

5.3 Canopy cover

When averaged throughout the season, Advena had significantly higher canopy cover than Kortessa (P<0.001). When focusing on the dates after the first fungicide treatment, the azole, SDHI, and the azole + strobilurin combination maintained their canopies for longer than the other treatments (P=0.05, **Figure 10**).

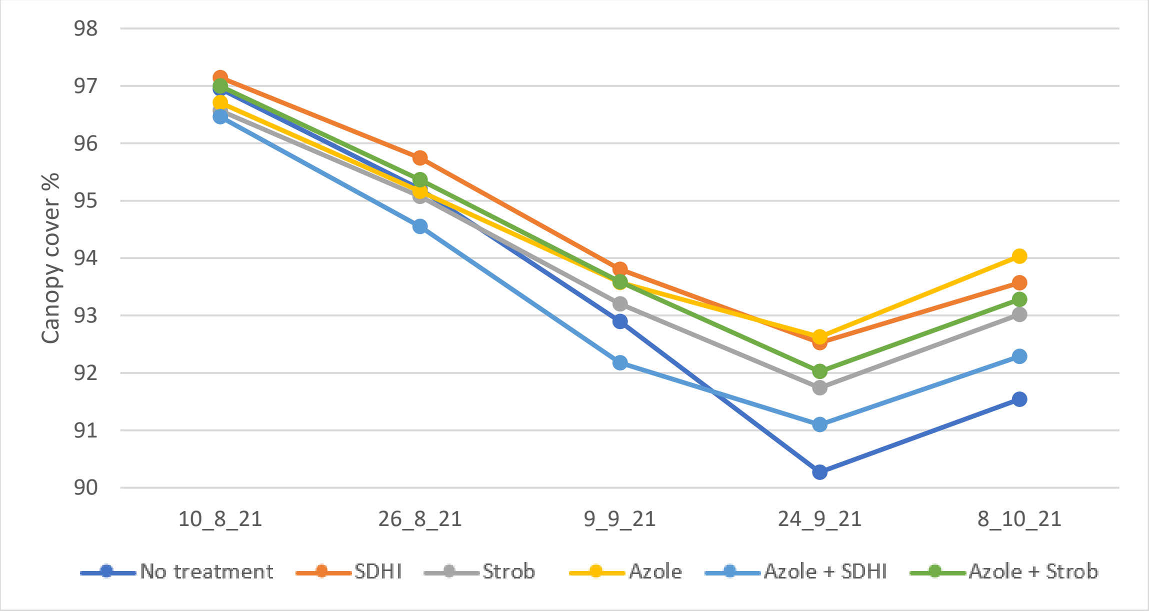

Figure 10. Comparison of canopy cover % in plots treated with various fungicide treatments, only including dates after initial treatment.

5.4 Crop Circle

5.4.1 NDRE

Using a repeated measures analysis, the lowest NDRE values were in the untreated control, and the highest values in the azole + strobilurin combination, the azole, and the SDHI (P=0.021, **Figure 11**).

NDRE gives an indication of the area of healthy vegetation, associating closely with chlorophyll content.

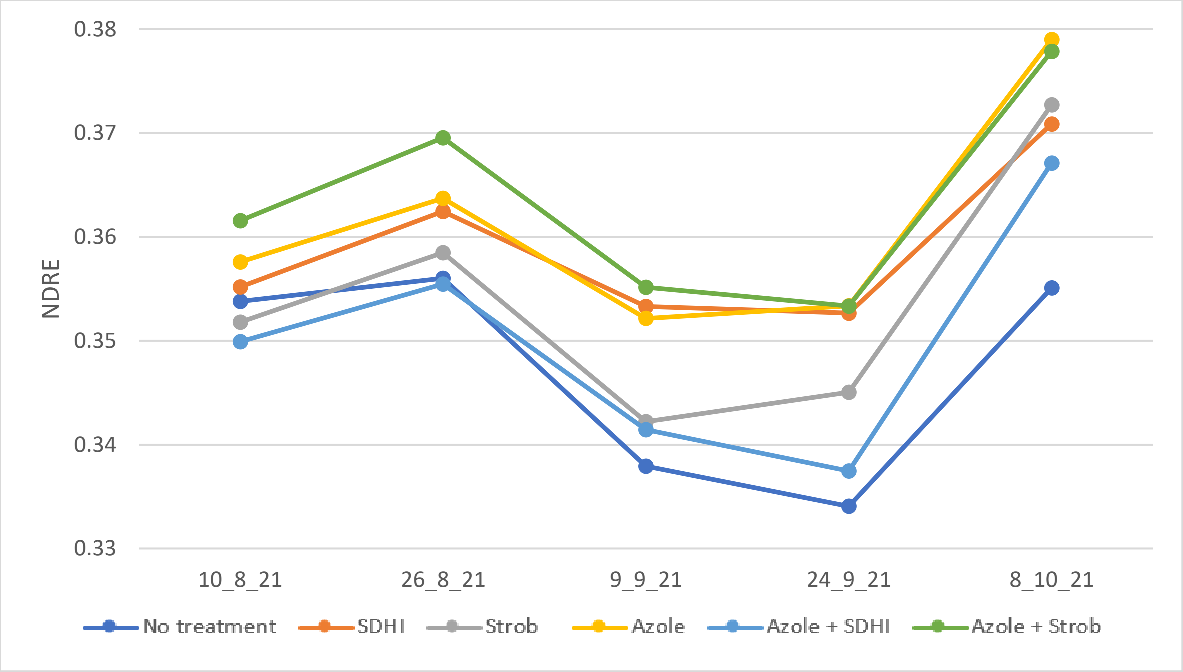

Figure 11. Comparison of NDRE between treatments over time, collected by the Crop Circle equipment.

5.4.2 NDVI

Repeated measures analysis found that the azole, SDHI and azole + strobilurin combination had higher NDVI than the other treatments (P=0.024, **Figure 12**). The strobilurin began to catch up with the other treatments later in the season. NDVI provides an estimate of green area from the canopy.

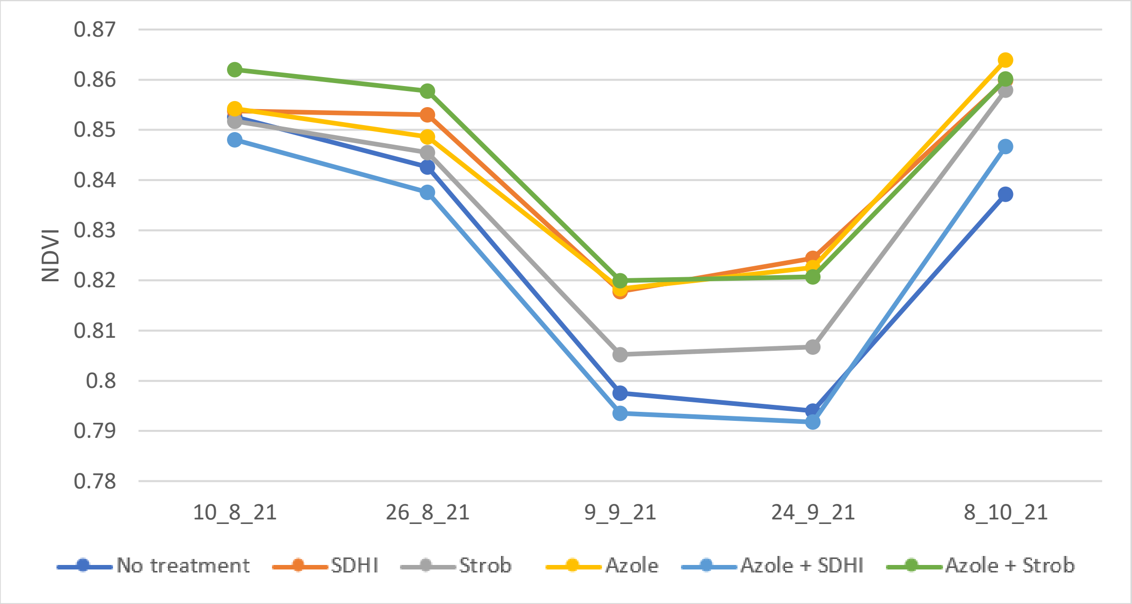

Figure 12. Comparison of NDVI between fungicide treatments over time.

5.5 Disease scoring

When comparing % rust in the plots, there were significant differences between fungicide treatments (P=0.001), between varieties (P<0.001), and there was an interaction between fungicide treatment and variety (P=0.026). Advena had much higher disease than Kortessa, and the most effective fungicide treatments were those that included an azole either by itself or in combination with others (**Figure 13**).

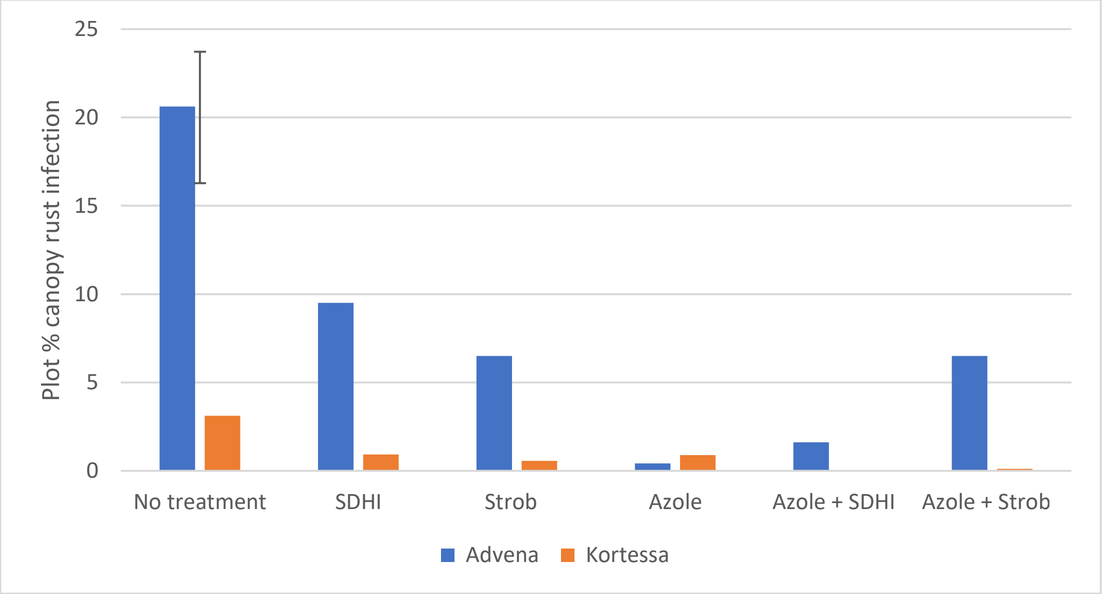

Figure 13. Interaction between treatment and variety when comparing rust % in the plots.

Powdery mildew was not seen in this year's field trial, so no scoring was carried out. Other diseases such as ramularia leaf spot were seen in minor amounts on a very small number of plots, so there was no benefit to plotting this data.

5.6 Final harvest

The difference between fungicide treatments was almost significant for leaf area, P=0.061, with the non-treated control having smaller leaf area than the treated plants (**Figure 14**).

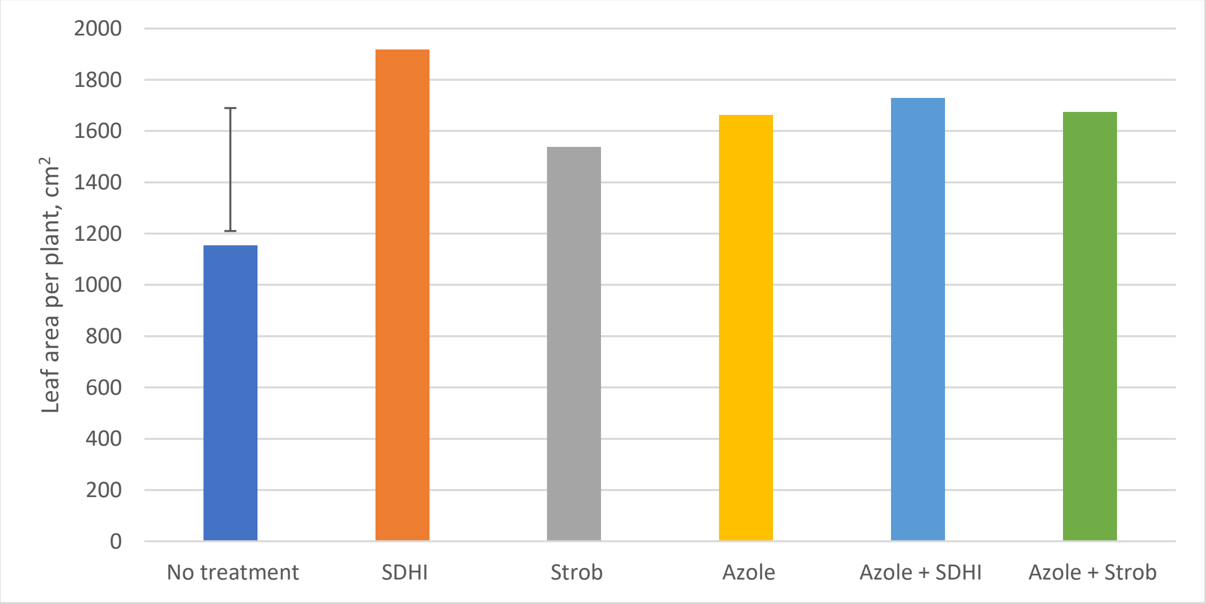

Figure 14. Comparison of leaf area per plant at harvest for the various fungicide treatments.

The azole + strobilurin combination had the highest *specific* leaf area (weight of leaf material per area of leaf, essentially leaf thickness), with the smallest being SDHI and azole treatments (P=0.05, **Figure 15**).

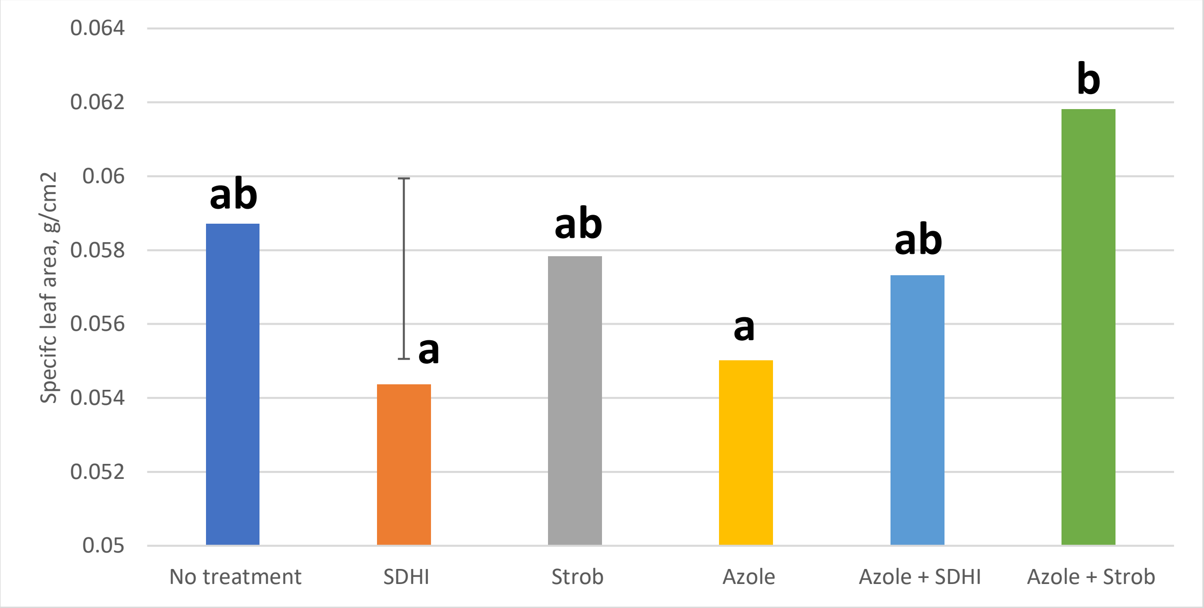

Figure 15. Comparison of specific leaf area between fungicide treatments, representing the biomass per unit of leaf area; the thickness of the leaves.

While there were no significant differences between treatments for fresh root weights, nor for sugar %, there was a significant difference between sugar yield; combining these two factors. The lowest yields were from the untreated control, and the highest yields came from the azole + strobilurin combination, the strobilurin, and the SDHI (P=0.042, **Figure 16**). Sugar yield was significantly higher in Advena than Kortessa, yielding 19.98 and 18.49 t/ha respectively (P<0.001) but there was no interaction between fungicide and variety.

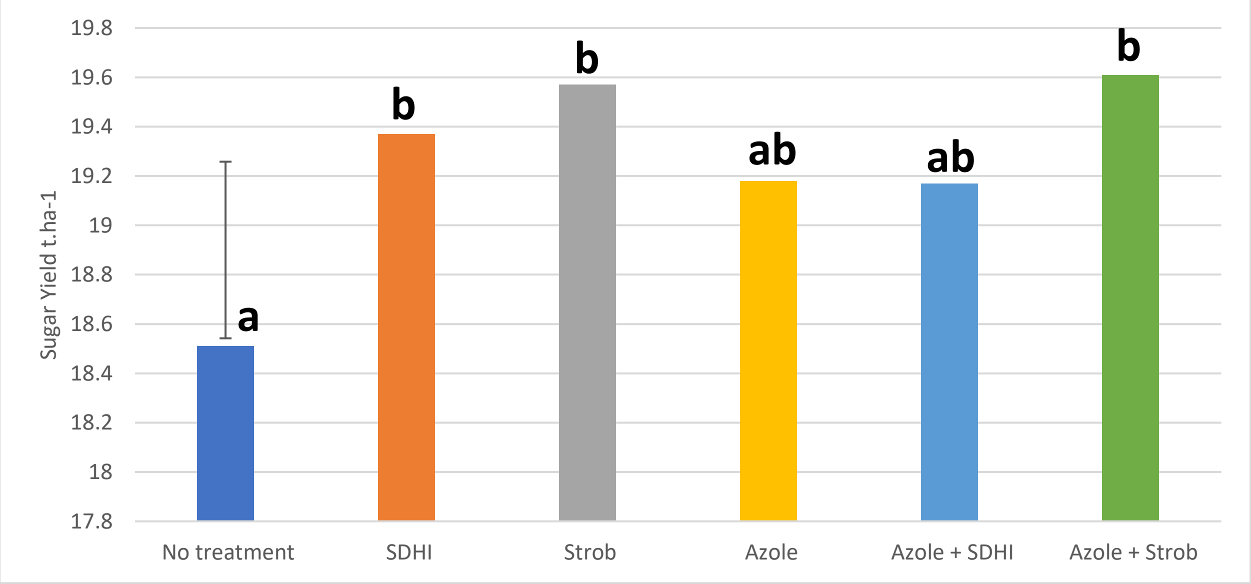

Figure 16. Comparison of sugar yield between fungicide treatments.

6. Discussion of first year data

The polytunnel trial overall showed the SDHI treatment to be most favourable in terms of root weight, and this was reflected in most of the physiological readings, including SPAD and leaf area. The strobilurin treatment mostly outperformed the untreated controls in physiological readings, but when it came to the yields, strobilurin treated plants were the lowest yielding. When root weight and sugar % were combined to find the sugar yield, there were no significant differences between treatments, although the order of highest to lowest was still SDHI > control > strobilurin, matching the order seen in root dry weight. The polytunnel experiment was small, and with only three reps, which made it harder to detect differences between treatments.

The effects of the fungicide treatments in response to drought were not as expected based on the literature, such as the studies into of the effects of strobilurins on droughted tomatoes, specifically with azoxystrobin (Giuliani et al., 2019) and with pyraclostrobin (Cantore et at., 2016), as well as the improved drought tolerance caused by kresoxim-methyl on droughted *Medicago truncatula* plants (Filippou et al., 2016); the general suggestion was that plants were more resilient to drought when treated with strobilurins, whereas this trial did not reveal the same relationship. All treatments led to a similar response under drought, and it was only under well-watered conditions that the treatments showed improved physiological activity. This unexpected response may potentially be due to sugar beet's ability to cope well in temporary drought (Barratt, 2021), so the impacts of the fungicides did not reflect those seen in other more drought-sensitive crops in the literature. This may be because those plants were benefiting from an improved general stress tolerance due to the strobilurin, rather than specifically improving their water use efficiency or avoiding droughting altogether. Examples of other forms of stress being alleviated by strobilurins in other crops are more abundant in the literature than drought stress, such as improved tolerance to transplanting injury and chilling stress in rice seedlings (Takahashi et al., 2017), reduced salinity stress in tomatoes (Boari et al., 2019) and in *Medicago truncatula* plants (Filippou et al., 2016) (note: this study found effects under both drought and salinity stress).

In the polytunnel, the LI-COR was only used on one date, on which the strobilurin outperformed the SDHI and non-treatment control for CO_2 assimilation. It would be interesting to repeat this data collection on a new trial where CO_2 assimilation is recorded throughout the season, in case different treatments perform better at different times through the year.

The field experiment was much larger than the polytunnel and this allowed for greater consistency across more treatments, which was reflected in the data. The azole + strobilurin combination, which is frequently used as standard in sugar beet, was consistently one of the highest performing treatments – so growers may have already been seeing many of the benefits of these physiological effects of fungicides for some time. Although the SDHI treatment had the largest leaf area, it had the lowest specific leaf area (**Figure 15**), suggesting that these larger leaves were thinner. The yields of the SDHI treated plants were lower than those of the strobilurin and the azole + strobilurin combination. These highest yielding treatments (strobilurin and the azole + strobilurin combination) were also those with the highest specific leaf area, though this relationship was not seen for the other treatments.

Where the strobilurin treatment was the lowest yield compared to the SDHI and non-treated control in the polytunnel experiment, it was interesting to see it yielding the highest out of those three treatments in the field. With the large scale of the field experiment, the yields are likely to reflect the true nature of these treatments compared to the polytunnel, but it’s still important to note that there could have been something of interest about the conditions in the polytunnel which led to these differing results.

Year 1 experiments opened up questions including the following:

1. How closely can SPAD represent true photosynthetic activity in the plants?
    a. Does this relationship change through the season? (i.e. if there is a visible ‘stay green’ effect, is it functional for photosynthetic activity later in season?)
2. What was causing the specific leaf area to differ between treatments?
    a. What specifically about the treatments was leading to thicker/thinner leaves?
3. Which reflectance indices are most closely linked to yield data? What do they represent?
    a. Does this point to the areas likely to be involved in yield changes?
4. How much of the yield differences can be attributed to disease pressure?

As well as some new questions which weren’t considered this year, including:

5. How do the different treatments affect nitrate reductase activity?
    a. Can they make the crop more efficient at using nitrogen fertiliser?
6. How do the treatments affect production of ethylene (especially later in season)?
    a. Can delayed senescence influence final yields?
7. Does the effect of treatments on photosynthetic activity change through the season?
    a. When is the important window of time to outperform the others for the highest resulting yields? (using the LI-COR for true photosynthetic activity)

These questions lead on to the current plans for the next experiments. There will be two main trials this season – a polytunnel trial and a field trial. The polytunnel trial will be focused on nitrogen uptake, nitrogen content in the leaves, nitrate reductase activity, and ethylene content in the leaves. Treatment will contain a strobilurin, as there is some evidence in the literature that strobilurins can increase nitrate reductase activity (Glaab & Kaiser, 1999) and reduce ethylene activity (Amaro et al., 2019) in other crops. There is a suggestion that one of the causes for this increased nitrate reductase activity is that a decrease in ATP production disrupts the activity of the proton-ATPases, affecting the pH gradient between the cytosol and the vacuole, and leading to acidification of the vacuole (Glaab & Kaiser, 1999). This lower pH is thought to activate an increase in nitrate reductase activity.

The remainder of this report is focused on the experimental design for the 2nd year of experiments.

2nd year experiments

7. Introduction

This year's experiments include a polytunnel trial and a field trial. The field trial is mostly a repeat of last year's, to add an extra year for repeatability. The polytunnel trial has a focus on the use of nitrogen fertiliser, and whether fungicides can help plants use fertiliser more efficiently. So far, the relationship between SDHIs and nitrate reductase activity has not been investigated, but this treatment will be included in the trial due to both SDHIs and strobilurins inhibiting mitochondrial respiration, and this could be one of the reasons for the increased enzyme activity. The specific cell-level processes which lead to these changes will be explored this year.

7.1 Nitrate reductase overview

Nitrate reductase is an enzyme found in plants which is involved in the process of nitrogen assimilation, by reducing nitrate into nitrite. It is found mainly in the cytoplasm of plant cells, sometimes found in small amounts on the outer envelope of chloroplasts. The enzyme catalyses the reduction to nitrite by transferring electrons from NADPH to nitrate and is the rate-limiting step in the nitrate assimilation process.

7.2 Strobilurin / nitrate reductase interaction

Nitrate reductase content has been seen to increase when spinach (*Spinacia oleracea*) leaf discs were treated with a strobilurin, specifically Kresoxim methyl (Glaab & Kaiser, 1999). It is suggested that the modulation of nitrate reductase activity could be due to changes in cytosolic pH caused by the action of the strobilurin – with artificial acidification leading to activation of the enzyme. A decreased pH in the cytosol also occurs in natural conditions such as during anaerobiosis, where ATP levels are much lower, leading to a decreased activity in proton-ATPases, and subsequent loss of maintenance of the pH gradient between the cytosol and vacuole. The suggestion is that the strobilurin temporarily leads to decreased ATP synthesis, and these conditions then lead to the acidification of the cytosol in the same manner as in the natural conditions mentioned above. It is suggested that this is the reason for the resulting increase in nitrate reductase activity and subsequent improved nitrate assimilation and yields.

This effect of increased nitrate reductase activity is also seen with artificial application of H_2O_2 (acidic) to the cell, but only up to a certain point, after which it became inhibitory to the enzyme's activity (Sharma & Shanker Dubey, 2005). This is useful to consider experimentally as it allows for the effects of the cytosolic acidification steps to be observed separately from the initial effects of the strobilurin, in order to determine which aspect of the strobilurin's application is leading to the increased nitrate reductase activity (i.e. first confirming that the strobilurin does lead to cytosolic acidification, then confirming if it is the acidification or something else causing the increased nitrate reductase activity).

7.3 Why SDHIs will be included

The trial will also include an SDHI treatment, in order to understand whether the processes affected by SDHIs in the plant also lead to increased nitrate reductase activity. There is a chance that the relationship found with strobilurins and nitrate reductase activity might be found with SDHIs too, because of the location of their mode of action. Both fungicide groups interfere with ATP synthesis, and the enzyme activity is suggested to increase as a result of decreased ATP synthesis. SDHIs interfere with a different point in the ATP synthesis pathway than strobilurins, so there is a reasonable chance that the treatment will not affect nitrate reductase, but it is similar enough to justify investigation.

Aims

i. **Polytunnel:** To investigate the effects of a reduced nitrogen fertiliser dosage on sugar beet while treated with an SDHI, an SDHI + azole combination, a strobilurin, or left untreated.
ii. **Field:** To investigate the effects of a large range of fungicides (including fungicide group combinations) on sugar beet physiology and resulting yields.

Objectives

i. **Polytunnel:** Compare plants treated with either an **SDHI**, a **strobilurin**, an **SDHI + azole** combination or left untreated under both standard nitrogen application and reduced nitrogen application.
    a. Compare physiological readings such as canopy greenness, chlorophyll content, canopy area, and a range canopy reflectance indices.
    b. Compare genetic and enzymatic measurements such as nitrate reductase activity, nitrate and nitrite content, and other factors associated with nitrate reductase and its upregulation.
    c. Compare biomass production and sugar yields.
    d. Compare disease presence and persistence.
ii. **Field:** Compare plants treated with either an **azole**, an **SDHI**, an **azole + SDHI combination**, an **azole + strobilurin combination**, a second **azole + strobilurin combination**, or left untreated.
    a. Compare physiological readings such as canopy cover %, canopy greenness, photosystem activity and a range of canopy reflectance indices.
    b. Compare biomass production and sugar yields.
    c. Compare disease presence and persistence.

Chapter C – Polytunnel experiment 2022

8. Methods

8.1 Experimental design

Variety

Kortessa (KWS) – low susceptibility to both powdery mildew and rust compared to others on the BBRO recommended list.

Treatments

Half of all boxes receiving standard nitrogen application (equivalent 120kg/ha), other half receiving no nitrogen (the soil in the polytunnel boxes is already quite high in N, so ‘reduced’ application is zero to attempt to widen the gap between the two N treatments)

Fungicide treatments as follows:

0. No treatment
1. Strobilurin
2. SDHI
3. Azole + SDHI

8.2 Data collection

8.2.1 SPAD

A SPAD chlorophyll meter is used as described in Chapter A of this report, once every two weeks.

8.2.2 Reflectance indices

An ASD fieldspec is used as described in Chapter A of this report, once every two weeks.

8.2.3 CO_2 assimilation and stomatal conductance

A LI-6800 portable photosynthesis system (LICOR, Lincoln, Nebraska, USA) is used to compare CO_2 assimilation and stomatal conductance between treatments.

8.2.4 Laboratory work

Enzyme assays will be carried out on leaf samples to determine the presence and activity of nitrate reductase between fungicide treatments. Chlorophyll extractions will be carried out to calibrate SPAD readings with true chlorophyll content later in the season, during the ‘stay green’ effect.

8.2.5 Harvest data

Plants will be harvested around October 2022. From each box, the two central plants will be collected for further analysis in the lab, including top weight, root weight, leaf area etc., while the outer 10 plants will be topped, and the roots sent to BBRO for sugar % and impurity analysis.

8.3 Data analysis

An analysis of variance (ANOVA) suitable for the experiment design is carried out using Genstat, using fungicide treatment and nitrogen application as factors, and including blocking in the analysis. Where significant differences are reported in the ANOVA, multiple comparisons are calculated using Duncan’s multiple range tests, with a confidence interval of 95%. Where data are collected over several weeks, a repeated measures ANOVA will be used to detect patterns over time. Graphs will then be developed in Microsoft Excel, using values of the least significant differences as error bars.

Chapter D - Field experiment 2022

9. Methods

9.1 Experimental design

Varieties

This experiment is shared with another student, with my main focus on all treatments rather than all varieties, therefore the full trial layout does not necessarily represent the selection of data collection for this project. In total, there will be four varieties:

- Kortessa (KWS), low susceptibility to foliar diseases*
- Advena (KWS), higher susceptibility to foliar diseases*
- BTS1140, medium susceptibility to foliar diseases
- Kortessa and Advena mix, alternate rows between high and low susceptibility to foliar diseases

*Varieties marked with an asterisk are the focus of my data collection.

The field was first treated on 29th July, and will receive another treatment when disease appears again.

Treatments

0. No treatment
1. Azole
2. SDHI
3. Azole + SDHI
4. Azole + Strobilurin **A**

5. Azole + Strobilurin **B**

Treatment 4 (azole + strobilurin **A**) is Escolta, a treatment frequently used on sugar beet crops, but due to a ban on cyproconazole this fungicide cannot be used after this year. Due to this loss of chemistry, a second azole + strobilurin combination will be included this year (Treatment 5) to calibrate the popular treatment against a newer one with the same fungicide type. Trials after this year will include only the newer fungicide.

9.2 Data collection

9.2.1 SPAD

A SPAD chlorophyll meter is used approximately once per two weeks using the same methods described in Chapter B of this report.

9.2.2 Reflectance indices

An ASD fieldspec is used once every two weeks in the field from June to harvest, using the same methods as described in Chapter B of this report.

9.2.3 MiniPam

The MiniPam may be used in the field this year, after training is carried out.

9.2.4 Harvest data

Plots will be harvested in November or December 2022 by removing 10 plants from each plot by hand for lab processing. The remaining plants in each plot will then be removed by BBRO using a beet harvester, before being sent for analysis at the tarehouse. The hand-harvested plants will be used for measurements including the tops of the plants and dry weights, whereas the machinery-harvested plants will be used in analysis of measurements such as sugar % and impurity content.

9.3 Data analysis

An analysis of variance (ANOVA) suitable for the experiment design is carried out for each of the measurements, as described earlier in this report.

10. Discussion

This year’s experiments aim to develop some further understanding of the physiological responses of sugar beet plants to a range of fungicides, as well as exploring the possibility of improved nitrogen fertiliser metabolism. There will be a deeper focus on more detailed physiological responses, using lab techniques to monitor enzyme activity and subsequent concentrations of nitrate and nitrite.

10.1 Improvements this year

Improvements to be made to this year’s experiments extending on from last year will include more regular disease scoring, so that it is possible to separate the effects of disease control from the other physiological effects leading to improved yields. The effects of disease on final yields can be compared between varieties in this year’s data, in order to provide some preliminary conclusions about this relationship before it is explored further in the next year of experiments. In addition to this, the LI-COR equipment will be used more frequently, to provide more reliable photosynthesis data, as opposed to relying only on indicators of chlorophyll content. More regular canopy photography will take place in the polytunnel experiment, and the scale of this trial will be larger than last year, using 4 replicates rather than 3. Both NDRE and NDVI were collected using the CropCircle last year and this will continue this year. Both of these measurements are helpful for assessing the green area of the crops, but NDRE using the red-edge section of the spectrum allows it to more accurately detect changes in chlorophyll content, and to accurately measure these changes later into the season. NDRE is often favoured too in crops with a more layered canopy, where NDVI may be saturated by only the top layer of crops and lead to less accurate measurements.

10.2 Literature focus

In terms of exploring literature this year, there is more focus on the effects of the fungicides on the plants at the cell-level. Much of the literature search will continue to be into the specific effects that fungicides have on things such as the ATP production pathway in mitochondria, and leading on to the effects of these changes on plant physiology. The mode of action for each fungicide type will be considered when looking at the cellular effects, as this may help to point at the specific pathways which are involved in both the fungicide action and the improved physiology. This year's experiments will essentially look more closely at the physiological changes seen and attempt to determine the specific interactions which lead to these physiological changes.

10.3 Backup for polytunnel experiment

Some fungicides do not penetrate further than the section of the leaf where they have been applied, so there may be issues with the ability for the fungicides to have an effect in specific areas within the plant. In the Glaab & Kaiser (1999) publication, they took leaf discs and submerged them in the fungicide mixture, rather than just treating whole plants. This may have allowed for the fungicide to reach the required areas within the plant for this interaction to take place. For this reason, if the polytunnel this year gives inconclusive results, an extended experiment will take place after this trial using the growth cabinets. Plants will be grown, and leaf discs will be taken and treated with the fungicides, before recording the effect on nitrate reductase. Although this will not provide a true representation of field responses, it could provide some insight into the cellular interactions of these fungicides. With the soil in my polytunnel boxes having such high nitrogen content, the results this year could be inconclusive, so in my 3rd year experiments (2023) it could be worth focusing on nitrogen usage in the field instead of boxes.

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