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

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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, ultimately reducing profits and discouraging some farmers from choosing to grow sugar beet. Two of the most significant foliar diseases for beet in the UK are rust and powdery mildew, which can reduce yields by up to 20%. Fungicides routinely used to treat foliar diseases on sugar beet have been observed to improve sugar yields even in cases of low disease pressure, suggesting activity other than 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. A combination of controlled environment, polytunnel and field experiments will be used to investigate the effect of a range of fungicide active ingredients on sugar beet growth and yield, in an attempt to quantify the physiological response of the crop to fungicides. Preliminary data with an initial controlled-environment experiment found some evidence of physiological response to fungicides in the absence of disease. However, the scale of the experiment and small number of replicates made it difficult to detect those differences. Subsequent larger trials could help to reveal the true nature of these relationships.

1. Introduction

1.1 Sugar beet crop introduction

Sugar beet (*Beta vulgaris* subsp. *vulgaris*) is a crop grown commercially for its production and storage of sugar. The concentration of sugar in the root varies depending on many factors including weather, fertiliser application, diseases and pests, but it is typically in the region of 15-18% of the fresh weight of the root (BBRO, 2020). A by-product of sugar beet cultivation is the pulp, which is the root matter once all sugar has been extracted, and this is generally used in animal feed. The sugar production is the commercially significant product so the efforts to improve yield focus on sugar production, not just root size alone. The crop is biennial, with root harvest in the first year of growth, placing importance on strategies to improve early establishment of the plant, emergence of leaf canopy, and optimal time before senescence must occur. The crop grows most successfully in the temperate zone, and is therefore a common crop for countries including the United Kingdom, France, Germany, the United States and Russia (FAO, 2020).

1.2 Optimising conditions

Sugar beet plants can respond to vernalisation at any point in their development, placing importance on sowing seeds late enough to avoid too much cold weather (Draycott, 2006). If young plants are vernalised then they will begin reproductive development before they have completed the vegetative stage, a process called bolting (Abo-Elwafa et al., 2006). When plants enter this stage, much of the sugar which would normally remain stored in the tap root is instead transported through the plant for use in the production of the seed head, reducing total yield. In the UK, sowing of this crop is in spring (typically in March, or early April in colder years), which is late enough to avoid plants bolting and early enough to allow the leaf canopies to intercept plenty of light through the growth period (Jaggard et al., 2009).

In the UK, roots are harvested anytime from September to March in a 'just in time' manner, so that roots can be transported to processing factories for sugar extraction with minimal time in storage where further yield loss can occur. The crop pulls a lot of nutrients out of the soil, so alongside issues like increased risk of root rot fungi and nematode infection, it is highly important to ensure crop rotation is correctly implemented (Melakeberhan et al., 2018). There should be a minimum of 3 years between sugar beet crops, so soils have the chance to replenish the required nutrients and also reduce the infection by beet cyst nematodes from previous sugar beet crops (Hauer et al., 2016). The cysts, which contain the eggs and hatched juveniles, can remain in soil for years, so the risk of re-infection if this rotation is not used correctly is extremely high (Eberlein et al., 2020).

For sugar beet crops to reach their potential yield, their leaf canopy must intercept as much radiation as possible. There is a linear relationship between total radiation intercepted and the yield of the crop. As mentioned previously, a major limitation to intercepting the most of the year's radiation is that the seeds cannot be planted earlier than March due to risk of bolting, so by the time the leaf canopy has emerged and grown, much of April, May and June's light radiation onto the crop has not been utilised to the full potential. This factor pushes importance on the rate of canopy expansion, to make the most of the available light at a given time. Two major factors which affect the rate of canopy expansion are the temperature and the uptake of nitrogen (Milford, 1985).

Uptake of nitrogen is important for the growth and expansion of the canopy, increasing the speed of canopy expansion and overall size of the final canopy. However, if plants are supplied with excessive nitrogen perhaps later in the season using 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. For an ideal sugar beet leaf area index (LAI) of 3, which will intercept 95% of incoming radiation, plants will require approximately 90-120kg N/ha.

A major limitation to sugar beet yield is water stress, which can lead to problems like delayed leaf appearance, slowed leaf expansion, decreased photosynthesis and accelerated senescence, which all lead to reduced light interception and lower sugar yields (Ober et al., 2010).

1.3 Common diseases of sugar beet

The crop is susceptible to various bacterial, fungal and viral diseases, as well as weeds, nematodes, and other pests. In the UK, there are two particularly common fungal diseases which tend to be the focus when developing fungicides for protection of UK sugar beet crops; these are powdery mildew (*Erysiphe betae*) and rust (*Uromysces betae*) (BBRO, 2019). Fungal diseases impact sugar beet growth, so treatment with fungicides is vital to retain – and even improve – the sugar yield.

1.3.1 Powdery mildew

Powdery mildew is one of the most commercially relevant fungal diseases which affect sugar beet crops in the UK. The disease is caused by the fungus *Erysiphe betae*. The fungus develops on the surface of the leaves, eventually leading to the yellowing and death of leaves. Preferable conditions for the onset of disease are dry, warm weather throughout daytime, with dew at night, which tends to be around July to October in the UK (BBRO, 2019). Infection with powdery mildew can reduce sugar yield by around 20% (Burks et al., 2011), so it is an important disease to combat as effectively as possible.

1.3.2 Rust

Rust is another highly commercially relevant fungal disease affecting sugar beet crops in the UK, caused by the fungus *Uromyces betae*. The fungus appears on both sides of leaves, as small orange-brown raised pustules, which can brush off onto machinery and passers-by in severe cases. Preferable conditions for onset of rust are periods of high moisture weather and temperatures between 15-20°C, which can often be from July onwards. Long periods of dew can increase the severity of the disease, and presence of the disease can make crops more susceptible to frost damage. The consequences of rust infection are not as detrimental to yield as powdery mildew, with yield reductions typically in the range of 2-5%, but in severe vases these reductions in yield can reach up to 14% (Hill, 2019).

1.4 Fungicides used to tackle disease

Various types of fungicides can be applied to crops as a means of decreasing the incidence of disease and subsequent loss of yields. Fungicides' abilities are limited to protecting uninfected plants and halting the spread from infected plants, but unlike fungal treatments for animals, these 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 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 throughout 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).

1.4.1 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.

1.4.2 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 encouraging resistance. For this reason, it is highly important to use strobilurins in combination with another product from a different fungicide group.

1.4.3 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.

1.5 Yield improvements

Strobilurins are often associated with improved growth and yield of crops, including tomatoes and soybeans. Studies into tomatoes have shown that strobilurin treatments improved total and marketable yield by as much as 19.1% (Cantore et al., 2016). The most significant improvements were seen when plants were under stress, with strobilurins improving plant water status under drought conditions. The specific strobilurin used in this experiment was pyraclostrobin, and this particular strobilurin appears abundantly in the literature as a treatment with yield-enhancing qualities. Another more recent study also looked at tomatoes but with a different strobulirin, azoxystrobin, which was also seen to improve the plant water status under drought conditions (Giuliani et al., 2019). The yield appeared to improve due to the suppression of ethylene.

Yield of corn has been shown to improve under treatment by pyraclostrobin, with improvements of up to 5% yield increase (Nelson and Meinhardt, 2011). Pyraclostrobin has been shown to sometimes have higher effectiveness at disease control and sucrose yields when applied with adjuvants compared to when applied alone. The effects of this fungicide are also commonly more pronounced when the plant is under stress conditions like drought.

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) reported 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).

In order to understand these improvements further, physiological interactions between the fungicide and the plant should be investigated, as well as clear comparisons between physiological processes with and without treatments, in the presence and absence of disease. As the timing of fungicide application is so critical to the overall success of the treatments, various timings should be used and evaluated throughout these investigations.

Some patterns of improved growth and yield with fungicides and other disease control treatments have been observed in wheat, and these improvements are shown not to be limited to disease control only. For example, fungicides containing the active ingredient prothioconazole are seen to improve wheat's tolerance to frost, which can be a major factor in yield loss, and other fungicides containing tebuconazole have been shown to improve root development (Anonymous, 2009). Another study in wheat concluded that pesticides containing imidacloprid improved plant stand, although some of these improvements could have been to wireworm control (Vernon et al., 2009). Another study investigated this same pesticide (imidacloprid) as well as clothianinidin, and concluded that these pesticides improved stress tolerance, both biotic and abiotic, by inducing salicylic acid-associated responses. This tolerance to both biotic and abiotic stresses could be a major factor in the improved growth and yield which was observed (Ford et al., 2010).

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 allows for a longer period of sugar production, 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).

The use of fungicides on crops has also been shown to be beneficial from a growth regulatory aspect, preventing crops growing too tall and causing them to lodge, which can lead to a significant loss of yield. This is especially relevant in crops which are top heavy and tall, like corn and wheat. As well as the growth regulation, strobilurins have been associated with improved stalk strength in corn (Wise and Mueller, 2011). Sugar beet do not have stems in the first year of growth, and the petioles are unlikely to be affected by the same mechanisms which improve stalk strength, but growth regulatory processes which affect the canopy in general could be interesting if they are seen in sugar beet too.

A possibility to consider when observing increased growth and yield in seemingly uninfected crops treated with fungicides is that the improved health may be due to the treatment 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 inoculated with the disease, but had not yet shown symptoms.

2. Methods

2.1 Cabinet settings

Three pelleted seeds (cv. Degas) were sown in each of 20 pots, and thinned out to one plant per pot once they had emerged. Initial cabinet settings were a 12 hour photoperiod with 1 hour dawn/dusk ramp, day temperature 12°C, and night temperature 8°C. After approximately 8 weeks, temperatures were increased to 20°C in the day and 14°C in the night to encourage growth. At this time of temperature increase, 0.551g of ammonium nitrate (0.19g of N) was applied to each pot, the equivalent of 50kg/ha in-field.

2.2 Treatment

Once the plants had 12 true leaves fully developed, they were sorted into blocks by their canopy area (area analysis carried out using photographs from above and ImageJ software). Due to space constraints, the 4 plants with the smallest canopy area were removed from the experiment before data collection began, leaving 16 plants remaining for the rest of the trial. Plants were placed in their size-determined blocks into each half of two controlled environment cabinets in case conditions differed at all between cabinets, shown below in **Table 1**.

Table 1. Summary of the plants and which treatment and block they receive, based on their area.

	Plant	Area (cm^2)	Treatment
1 LHS	7	907.6	4
	13	912.8	2
	17	954.4	1
	4	981.9	3
1 RHS	12	983	4
	11	986.1	3
	19	1002.2	2
	20	1027.2	1
2 LHS	18	1033.4	1
	6	1049.4	4
	10	1063.7	3
	2	1064.4	2
2 RHS	15	1077.5	2
	5	1123.8	3
	1	1150.1	1
	8	1162.7	4

Plants were treated with 4 treatments, outlined below:

1. Azole and strobilurin
2. Azole only
3. Strobilurin only
4. No treatment control

2.3 Data collection

One week after treatment, physiology-based data collection began. Once weekly, a Minolta SPAD meter was used on the newest fully emerged leaf of each plant to determine greenness as an indication of chlorophyll content. One leaf was chosen for each plant and 3 readings were taken per leaf for an average. Also once weekly, a Fluorpen was used to determine Quantum Yield of the leaves. The newest fully emerged leaf was chosen as the one leaf per plant, and 3 readings were taken from each leaf for an average. The final weekly data collection was the measurement of leaf reflectance using the ASD FieldSpec. In the first week after treatment, only the newest fully emerged leaf was selected for data collection, but from the second week onwards, both a newly fully emerged and an old (but not yet visibly senescing) leaf were chosen for data collection. For each leaf, only one reading per leaf was taken. Photographs were taken from above 4 and 6 weeks after treatment using a DSLR camera (Canon EOS 1100D 12MP), and processed in ImageJ to determine canopy area.

2.4 Harvest

Plants were harvested approximately 8 weeks after treatment. The whole plant was washed, before separating the root from the leaves and weighing separately the roots, petioles and leaves. Dead leaves which were discoloured and dry on the fresh plant were weighed separately from the main selection of leaves. All leaves (now with petioles removed) were placed in a leaf area meter (LICOR LI-3100C) to determine true leaf area. Dead leaves were not included in this area analysis. After all fresh weights were recorded, all components of the plants were placed in a drying oven for later recording of dry weights. Leaves, petioles, and dead leaves were measured after 3 days in the drying oven, and roots were measured after 7 days in the drying oven.

2.5 Data processing

2.5.1 Reflectance data

The ASD FieldSpec outputs a large dataset containing reflectance data at each of a large range of wavelengths. Using these outputs, a selection of reflectance indices can be calculated. The indices selected in this trial were anthocyanin ratio (ARI), blue green pigment index (BGI), carotenoid reflectance index (CRI), normalised difference vegetation index (NDVI-1), normalised water index-1 (NDWI-1), ratio analysis of reflectance spectra of chlorophyll a (RARSa), ratio analysis of reflectance spectra of chlorophyll b (RARSb), water index (WI), red edge NDVI (rNDVI), and mNDblue.

2.5.2 Canopy area

The photographs from above were processed in ImageJ using specific colour threshold values and a set scale to determine the area of each plant's canopy. The colour thresholds depend on the lighting & background etc. so these were manually set each time using the first photograph in each folder as a reference point. A small plastic object 1.2cm wide was used as a size reference for all photographs. The ImageJ output is a list of the files with their corresponding canopy areas in cm^2.

2.5.3 Harvest data

To gain a better understanding of the canopy growth which includes the combination of area and weight rather than viewing each aspect independently was carried out by calculating leaf mass per area (g/cm^2).

2.6 Data analysis

All datasets (physiology & harvest data) were imported into Genstat and analysis of variance was carried out on each. For SPAD, Quantum Yield, and reflectance data, there were several weeks of measurements so repeated measures ANOVAs were carried out after the initial ANOVAs. For any cases where significant relationships were observed between treatments and physiological data, multiple comparisons were used to determine where the relationship was found. For these cases, Fishers least significant difference (LSD) procedure was used. RStudio was used to generate box plots to compare values between treatments at each week of data collection.

3. Preliminary Results

3.1 SPAD

There were no significant differences found between treatments for SPAD readings at any date, nor were significant differences seen when a repeated measures ANOVA was used. Results were highly variable, in part due to the high variability of SPAD data but also due to the small sample size. This high variability can be seen below in **Figure 1**.

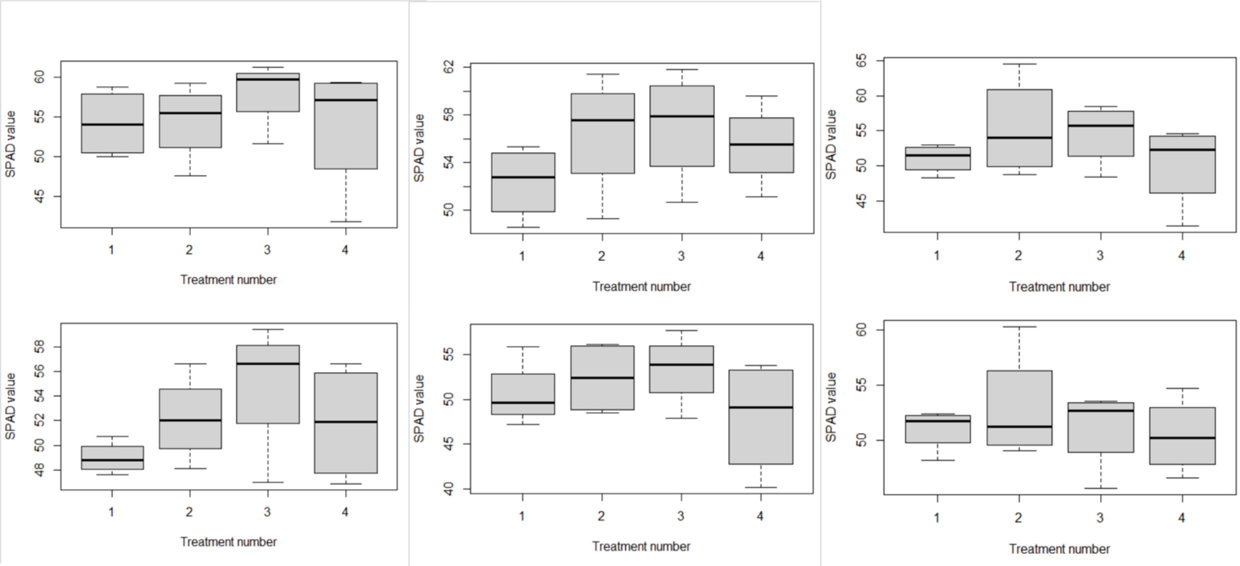

Figure 1. SPAD readings between treatments, on weeks 1 to 6 after treatment application.

3.2 Quantum yield

Similarly to the SPAD results outlined above, the Quantum Yield results (recorded using the Fluorpen) were highly variable and no significant differences were found between treatments. This was also true when a repeated measures ANOVA was used. This high variability can be seen below in **Figure 2**.

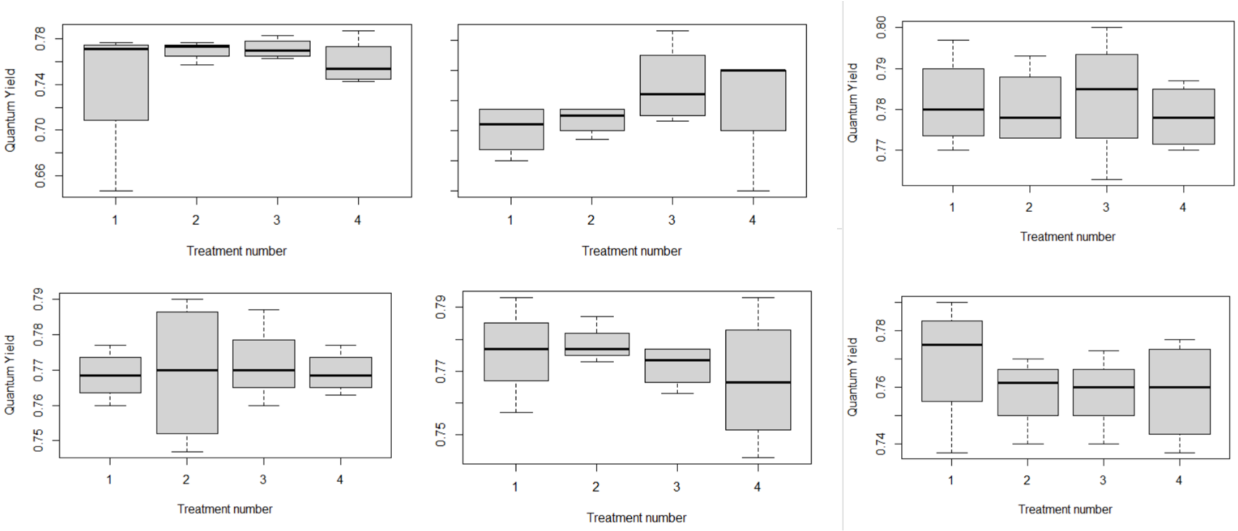

Figure 2. Quantum yield between treatments, on weeks 1 to 6 after treatment application.

3.3 Reflectance indices

Very few of the reflectance indices differed between treatments, but there were some exceptions. Blue green pigment index (BGI) differed significantly between treatments on week 1 after treatment, with the control having a higher BGI reading than all the other treatments (p = 0.018, **Figure 3**). However, this difference was not evident at any of the other sampling points.

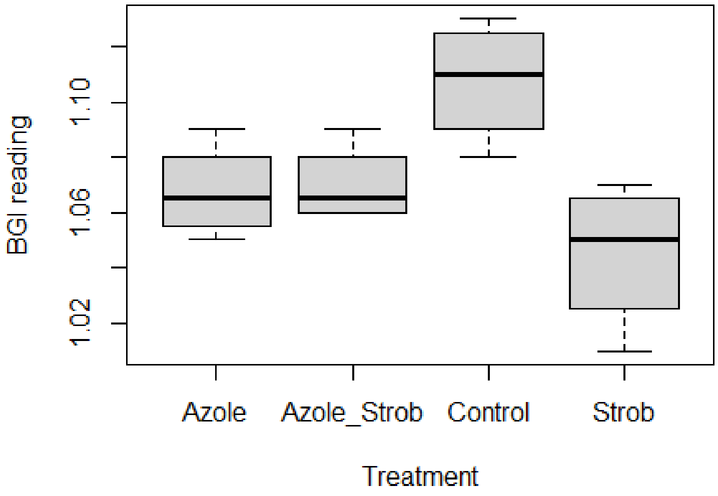

Figure 3. Relationship between treatments applied and BGI reading on week 1 after treatment.

When a repeated measures ANOVA was used, ratio analysis of reflectance spectra of chlorophyll b (RARSb) was higher when treated with an azole or strobilurin alone, but lower when they were used in combination or not used at all (p = 0.03, **Table 2**).

Table 2. Relationship between treatment applied and RARSb reading.

Azole	Strobilurin	Azole + strobilurin	Control
12.84	12.86	10.79	10.78

Further to this, using the repeated measures ANOVA, carotenoid reflectance index (CRI) was very close to being significantly different, where the azole treatment alone resulted in the highest CRI value, with the control being the lowest (p = 0.056). The combination of both fungicides resulted in a lower CRI than when they were each used in isolation. A larger sample size would help to determine if this relationship exists or if it was due to chance. This is displayed in the table below, **Table 3**.

Table 3. Relationship between treatment applied and CRI reading.

Azole	Strobilurin	Azole + strobilurin	Control
10.25	9.45	8.59	7.93

3.4 Canopy area

There were significant differences in canopy area between treatments at 4 weeks post-treatment with strobilurin treatment alone resulting in a larger canopy area than the other treatments (p = 0.030), but not at 6 weeks post-treatment (p = 0.758). This is displayed in **Figure 4** below.

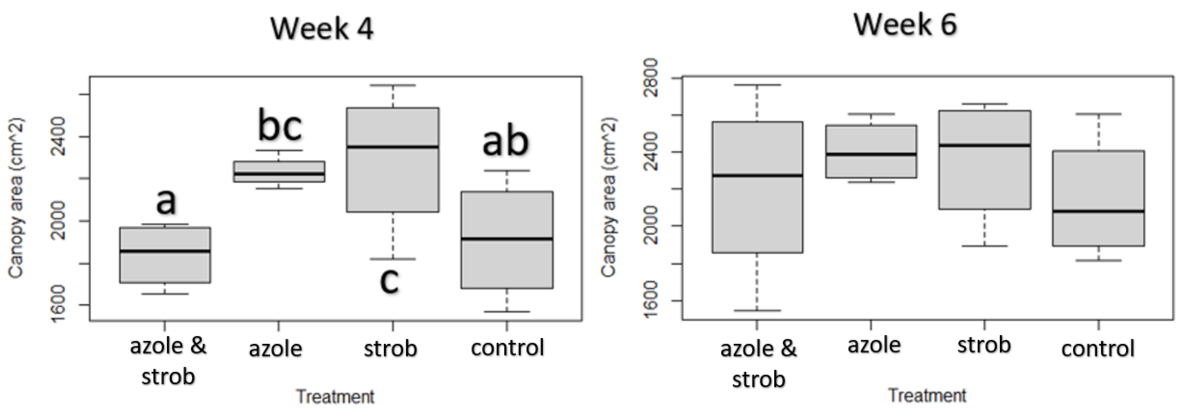

Figure 4. Relationship between treatment and canopy area at 4 and 6 weeks post-treatment.

Due to this difference at one point in time and lack of difference later on when plants were reaching harvest date, it emphasises the need to take these photographs more frequently in subsequent trials.

3.5 Harvest data

The yield data did not differ between treatments, and was highly variable for each component. Some of the leaf information (leaf area, petiole dry weight, leaf dry weight) had a visible suggestion of a relationship when viewed on a plot, with strobilurin only being higher than the others, but this was not statistically significant. These plots are displayed below, in **Figure 5**.

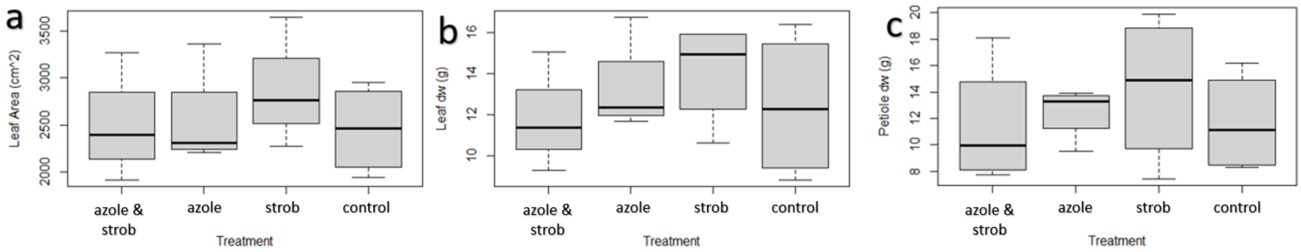

Figure 5. Relationship suggested between treatments and leaf yield data, leaf area *(a)*, leaf dry weight *(b)* and petiole dry weight *(c)*, not statistically significant but worth relating back to in future experiments.

4. Discussion

4.1 Overview

This initial small scale controlled-environment experiment provided a means for a range of training techniques and an opportunity to begin planning future experiments. Many of the results were not statistically different between treatments, but this is not surprising due to the size of the experiment, and the subsequent larger experiments planned for this project could help to reveal the true nature of relationships between treatments and plant physiology.

4.2 Physiological results

4.2.1 SPAD and QY

Both the SPAD and Quantum Yield data was highly variable and no statistically significant differences were seen between treatments. Looking at the graphs regardless of statistical significance, there were still no clear patterns of any particular treatment performing better or worse than any others throughout the trial. These techniques will be carried forward into the subsequent polytunnel and field experiments, but the much larger scale to the experiment could help to reveal any relationships between treatments and these physiological readings, if such relationships exist.

The SPAD reading gives an indication of greenness – providing a non-destructive method of estimating chlorophyll content of leaves. The readings can be highly variable as standard, so it is important to attempt to reduce this variability as much as possible. The larger scale experiments should help, and there is also importance in taking the readings early in the morning, as readings can become an unreliable estimate of chlorophyll content during the afternoon. The high variability is also common in the Fluorpen, so readings for subsequent experiments will be taken in the mornings for both of these techniques in an attempt to reduce the spread of data.

4.2.2 Reflectance indices

Using the FieldSpec produces a plethora of reflectance data from which reflectance indices can be calculated. A selection of 10 different indices were used, concerned with a variety of physiological conditions such as chlorophyll content and water content. Though these were helpful to look at, a larger selection of indices would open up more options for analysis and potentially reveal some relationships which weren't covered by this initial selection. The subsequent trials will take into account a much larger selection of reflectance indices, based on those which appear in the literature to be affected by stress, treatments, and resulting changes in yield.

The only reflectance index which had a significant difference between treatments was BGI in week 1 after treatment, with the control being higher than all 3 other treatments. To check if the relationship continued (albeit non significantly), further weeks were checked but this was not seen. Due to this one-off significant difference, this reflectance index will be used in subsequent experiments. Using repeated measures analysis, RARSb was revealed to have a significant difference between treatments. The azole and strobilurin used in isolation each had higher values than the control and the combined fungicide, suggesting that each of the components increase chlorophyll b content but this effect is dulled when the two are used in combination (which they often are, in practice). The repeated measures analysis also drew attention to one index which was close to having a significant difference between treatments, CRI. The value was highest for azole only, followed by strobilurin only, then the combination, with the control being the lowest value. This is the second relationship seen where the fungicide types used in isolation perform better than in combination, which could become highly interesting if this trend is observed in larger trials too. With a P value of 0.056, this index could be of interest in the subsequent experiments, so this will be included in the next selection.

4.2.3 Canopy area

A larger canopy area allows for more light interception, a key component in improving yields. It is therefore desirable for the canopy to grow quickly, and persist at a large size for the duration of the biomass production period. The plants treated with strobilurin only had a larger canopy area than the azole & strobilurin combination, as well as the non-treated control. The plants treated with azole only were larger than the combination, but not significantly larger than the negative control. It is interesting that the relationships observed in the leaf reflectance data are similar to those observed in canopy area – with the individual components of the fungicides improving physiological attributes more than their combined use. The canopy area at 6 weeks after treatment was no longer significantly different between treatments, which is not surprising as at this point the plants had many senescing leaves and new growth of the canopy was no longer occurring. Due to the interesting relationship at 4 weeks, this highlights the importance of taking photographs every week, rather than a select few. Knowing when these relationships occur can help to identify the reasons for their causes and their impacts on the productivity of the plants.

A useful way to look further into the productivity related to canopy area is to take into account the weight of the leaves, so as not to falsely conclude that larger thinner leaves guarantee higher productivity than slightly smaller but thicker leaves. To investigate this, specific leaf area was calculated, by dividing the leaf area by the leaf dry weight. While this didn't reveal any further relationships in this trial, it is a helpful calculation which will be used when processing the yield data in subsequent experiments.

4.2.4 Harvest data

Much of the harvest data was not statistically different between treatments, however when looking a the boxplots of the data, some mild trends did appear to be emerging with data about leaf area. With plans treated with strobilurin only, the leaf area was slightly larger than other treatments, so the larger trials following this initial experiment could help to reveal if this relationship truly exists. Many more plants per treatment will be harvested in the subsequent trials so if such a relationship exists, it is far more likely to be detected in these.

5. Conclusions

This experiment was useful for gaining training and experience with the sugar beet crop and lab facilities, and the subsequent experiments will incorporate these findings into their design. There appears to be importance on using fungicides types in isolation as well as in combinations, so this will continue in future experiments.

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