INVESTIGATING THE PHYSIOLOGICAL EFFECTS OF FUNGICIDES ON SUGAR BEET GROWTH AND YIELDS

By

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Abstract

Sugar beet (*Beta vulgaris* subsp. *vulgaris*) is susceptible to a range of foliar diseases, which can damage the plants and result in significant yield losses. Fungicide programs are routinely used in sugar beet cultivation to gain control of the major foliar diseases and protect plant yields. Some fungicides have been observed to improve the physiology and yields of crops, even in conditions where disease is not present, but this relationship has not yet been determined in sugar beet. This project investigated the relationship between fungicides and sugar beet physiology and yields, to determine the physiological reasons that this process may be occurring.

A combination of field, polytunnel and glasshouse experiments was used to investigate the responses of sugar beet plants to a variety of conditions, with a selection of fungicides. The overarching themes were comparing responses to fungicides of contrasting modes of action under droughted conditions, various nitrogen fertiliser levels, and standard field conditions with varieties of differing disease susceptibility. Measurements were taken which included leaf photosynthetic activity, spectral reflectance, canopy cover, and yield data including leaf weight, root weight, and sugar yield. Field experiments were conducted in the presence of disease, but the amount of disease for cercospora leaf spot and leaf rust were relatively low.

Across most of the experiments, the fungicides which tended to show increased yields and more positive physiological responses (and which couldn't be exclusively attributed to visual biocidal effects of the active ingredient) belonged to the groups of azole + strobilurin combination, and azole + SDHI combination. In the field experiments in particular, the azole + strobilurin fungicide frequently had better trait scores than other treatments, including the no fungicide control, and also resulted in the highest sugar yields. The no fungicide control was frequently the poorest performing treatment, although the reasons for the lack of consistent response across different experiments were unclear.

For the experiments that focused on responses under abiotic stress, namely drought stress and nitrogen limitation, the effects of these stress conditions were highly significant on plant physiology and yields, while the effects of fungicides tended to be more apparent while conditions were otherwise favourable.

There were indications that some fungicides may be effective at reducing the normal stress responses of the plants under abiotic stress, which allowed for more photosynthetic activity to take place even when conditions were not optimal, which subsequently increased yields.

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Table of contents

List of abbreviations

Abbreviation	Description
°C	Degrees Celsius
µmol	micromole
ABA	Abscisic acid
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BBRO	British Beet Research Organisation
BChV	Beet Chlorosis Virus
BMYV	Beet Mild Yellowing Virus
BYV	Beet Yellows Virus
cm	centimetre
CO_2	Carbon dioxide
DSLR	Digital single-lens reflex camera
DW	Dry weight
$FADH_2$	Flavin adenine dinucleotide
FW	Fresh weight
g	grams
ha	hectares
Kg	kilograms
L	litres
LAI	Leaf area index
LC-MS	Liquid chromatography mass spectrometry
LSD	Least significant difference
m	metres
mg	milligrams
min	minutes
mm	millimetres
mol	mole
N	Nitrogen
nm	nanometres
NPQ	Non-photochemical quenching
QoIs	Quinone outside inhibitors
QY	Quantum yield
RUE	Radiation use efficiency
s or sec	seconds
SDHI	Succinate Dehydrogenase Inhibitor
SE	Standard error
SOD	superoxide dismutase
SPAD	Soil Plant Analysis Development
WUE	Water use efficiency

List of equations

Index	Description	Equation
BGI	Blue green index	R450/R550
CRI	Carotenoid reflectance index	(1/R510)-(1/R550)
MnDBlue	Blue-band modified normalised difference index	(R440-R728)/(R440+R850)
NDRE	Normalised difference red edge index	(R842-R717)/(R842+R717)
NDVI	Normalised difference vegetation index	(R800-R680)/(R800+R680)
NDWI	Normalised difference water index	(R970-R900)/(R970+R900)
NPCI	Normalised pigment chlorophyll ratio index	(R680-R430)/(R680+R430)
RARSb	Ratio analysis of reflectance spectra chlorophyll b	(R877/(R650*R700)
TrVI	Triangular vegetation index	60(R670-R550)-100(R670-R550)

R = measured reflectance at given wavelength (nm)

List of tables

List of figures

Chapter 1. General introduction to sugar beet and the effects of fungicides on plant physiology

1.1 General introduction to sugar beet crop

Sugar beet (*Beta vulgaris* subsp. *vulgaris*) is a crop grown across temperate climates for its production of sugar. The crop is grown in areas which are unfavourable for the production of sugar cane, which is exclusively grown in tropical and subtropical zones. In 2022, the countries with the highest sugar beet production were Russia, France, the USA, Germany and Turkey (Food and Agriculture Organization of the United Nations, 2023). According to the same source, the UK was the 11th highest sugar beet producing country in 2022, with over 6 million tonnes produced.

Sugar beet is generally cultivated as a break crop within a rotation of other more intensively farmed cereal crops, providing an opportunity to reduce the risk of disease of these crops, and reduce the pressure of pest and weed levels. Choosing a good rotation of crops with sufficient diversity is an important factor in improving the health of farmed land. Although sugar beet is used as a break crop, it has a high economic significance. UK sugar beet fulfils around 50% of the UK's demand for sugar, as well as exporting sugar globally, which supports up to 9,500 British jobs (British Sugar, 2018).

In the UK, sugar beet is primarily grown in East Anglia and the East Midlands, where four sugar beet processing factories are located. This close proximity of growing areas and sugar processing factories means that the total distance travelled by sugar beet is minimised, at an average of 28 miles from field to factory (British Sugar, 2018). The locations of these sugar beet processing factories are displayed in **Figure 1.1** below, created by NFU (2021).

Figure 1.1. Locations of sugar beet processing factories in the UK; Newark, Wissington, Bury St Edmunds, and Cantley. Peterborough noted on the map as the British Sugar headquarters (NFU, 2021).

The sugar beet plant consists of a white taproot with a flat crown, and a rosette of leaves. Sugar is produced in the leaves via photosynthesis, after which it is stored in the root. Sugar beet is a biennial plant sown in the spring, which spends the first year of growth accumulating sugar and expanding the tap root, and the second year of growth entering a reproductive phase. Sugar beet is harvested at the end of the first year, before the plant enters reproductive growth, while the sugar content in the root is highest. Modern practices and the emergence of tailored sugar beet varieties have increased the average sugar content in roots to reach up to around 20%. This value tends to be lower in seasons where weather or disease caused significant impacts on growth and productivity. The remainder of the root consists of water (approx. 75%) and pulp (5%). The pulp can be used for other products, including animal feed, which reduces waste produced by the sugar beet industry, as well as improving value of the crop as a whole.

1.2 Limitations to optimal growth

Sugar beet yield is directly proportional to the light intercepted, which places importance on sowing as early as possible, increasing the speed of canopy expansion, and improving canopy cover and persistence throughout the season. The factors which can interfere with this ideal resource capture and sugar accumulation are explored in this section.

If the crop is sown too early in the year, the plants are at risk of bolting, a result of vernalisation, which is where the crop enters the reproductive stage in the first weeks of growth, instead of going through vegetative growth for the year. This process occurs because the plant would normally respond to cold temperatures as a signal that the first season has ended, and it is now time for flowering and reproductive growth as the second season. If the plants receive this low temperature signal shortly after sowing, they will start reproductive growth, and this results in a tall flowering plant which is not focused on accumulating sugar in the tap root. The processes of vernalisation and de-vernalisation are mechanistically complex, but are well understood from a production standpoint. This process is detrimental to sugar yields, so growers have to ensure that sowing date is late enough in the year to avoid this risk. In the UK, this tends to mean that sugar beet crops are sown in March and April. Prolonged periods of temperatures below 12°C increase the risk of bolting (Jaggard et al., 1983), meaning that a couple of cold days will not usually result in bolting, but extended durations at this temperature will increase the risks.

There are many factors which can impact the rate of emergence and canopy expansion in the early stages of sugar beet growth. It is widely suggested to sow around 1.25x the ideal plant population, to account for losses through failed emergence. Yields are optimised when plant population is approximately 100,000 established plants per hectare. Soil preparation is also key to improving emergence, with current advice indicating that preparing the seed bed to a depth of 5-7cm is ideal. For the same reason, it’s important to avoid compaction wherever possible, as compacted soils can significantly impact emergence. After sowing, early seedlings can be at risk of grazing pests, including birds and small mammals. Aphid risk can also be high at the very beginning of the season, depending on many factors which contribute to the risk of early flight, such as temperatures and rainfall in early months of the year.

During the middle stages of the season, where the canopy has closed and has not yet started to decline or senesce, there are other factors which can affect the canopy. Aphids which may have entered the crop at a much earlier growth stage may have been carrying viruses that cause virus yellows, the effects of which will begin to be visible at this stage. The virus yellows are a selection of different viruses which lead to physiological changes in the leaves, and can have subsequent detrimental effects on sugar yields. These viruses and their impacts are explored later in this introduction. Another major factor which can impact canopy health and subsequent yields is fungal foliar diseases. In the UK, the major foliar diseases which pose a risk to yields currently are Cercospora leaf spot (*Cercospora beticola*), rust (*Uromyces betae*), and powdery mildew (*Erysiphe betae*). Each of these diseases are explored later in this section. Another factor which can have a significant impact on the canopy is weather; where long periods of drought and warmer weather can cause leaves to wilt. Additionally to the decreased light interception of a wilted canopy, there are effects including a shut down of photosynthetic functions, and wilted leaves touching the hot soil for extended periods which can damage the leaves and lead to canopy area loss. Later in the season, periods of freezing temperatures can lead to canopy loss, especially if the leaves are already damaged by foliar disease. While much of the light interception and sugar production will have already occurred by this stage, a fuller canopy can insulate the crop, meaning that a frost damaged canopy can, in turn, be less effective at protecting the crown of the root from freezing. This effect can speed up degradation of the root and lead to yield losses.

1.3 Impacts of virus yellows

Peach potato aphid (*Myzus persicae*) is a major pest of brassicas, and while it doesn't tend to feed on sugar beet long term, they can transmit viruses from any initial feeding on the leaf of young sugar beet plants. The aphids act as carriers for a range of plant viruses, which tend to sit in the stylet (mouth part) of the aphid. Younger sugar beet plants are more susceptible to significant damage resulting from aphid presence, with the plants gaining more substantial protection around the 12-leaf stage. In the UK, it is at this 12-leaf stage where the threshold for spraying insecticide increases. After the 16-leaf stage, spraying is no longer required.

The virus yellows are a complex of three major viruses which affect sugar beet: Beet Mild Yellowing Virus (BMYV), Beet Chlorosis Virus (BChV) and Beet Yellows Virus (BYV). BYV causes the most detrimental effects of the three viruses, with yield losses of up to 50% in severe cases. BMYV can lead to yield losses as high as 28% and BChV has been seen to reduce yields by 22% (British Sugar, 2023), highlighting the importance of controlling all three of these viruses. Each of these viruses result in various yellowing of the leaves, and can lead to the leaves becoming thicker and brittle.

Early detection and treatment of aphids is vital for reducing the impact of viruses, and the BBRO (British Beet Research Organisation) recommend searching for aphids in the heart of the early canopy, as well as on the underside of each leaf. Since the ban of neonicotinoid seed treatments, these particular chemical interventions can only be used if an emergency use is approved. This has increased the pressure of these aphids and as such there has been more importance placed on other methods of protecting the crop. This includes improving farm hygiene and biosecurity, by ensuring there is no infected beet material left on farm between seasons, and by being aware of which crops and vegetation can serve as an overwintering host for the virus.

1.4 The major fungal foliar diseases of sugar beet

Up until 2020, the major foliar diseases which posed a threat to sugar beet crops in the UK were rust (*Uromyces betae*), and powdery mildew (*Erysiphe betae*). Cercospora leaf spot (*Cercospora beticola*) has been an issue for sugar beet crops across Europe for many years, but didn't pose as a recurring threat to UK crops until recent years. Below is a summary table of the three major fungal foliar diseases affecting UK sugar beet crops (**Table 1.1**).

Table 1.1 Summary of the three major fungal foliar diseases affecting UK sugar beet crops. All photographs produced by BBRO.

Disease name	**Rust**	**Powdery mildew**	**Cercospora leaf spot**
Pathogen name	*Uromyces betae*	*Erysiphe betae*	*Cercospora beticola*
Photo			
Features	Small raised pustules, orange/red, later turning brown.	Powdery white or light grey growth covering the leaves.	Small brown lesions with red/brown border, expanding to light grey

			middle with very small black spot at centre.
Yield loss	Up to 14%	Up to 20%	Up to 50%

In 2020, there was a compounded effect seen by the combination of severe virus yellows and severe cercospora infection, which resulted in significant yield losses across the UK growing area. Warmer weather in the UK in recent years has added to these risks, with cercospora thriving in conditions with warm temperatures and high leaf wetness.

There is a pressure on fungicidal control to adapt to this emerging threat, as the traditional treatments in the UK are less effective at controlling cercospora than they are at controlling rust and powdery mildew. Specifically, cercospora seems to be generally resistant to strobilurins, and triazoles have been less effective at treating cercospora than other foliar diseases. UK growers have had to adapt to this new disease pressure by using different fungicide programmes. As this disease has been a frequent threat in much of Europe for some time, the UK has been able to observe how the disease is controlled elsewhere, including different fungicide products and beginning to choose varieties which show more resistance than others.

1.5 The use of fungicides in sugar beet

As sugar beet are susceptible to a range of foliar diseases, it’s standard practice for growers to treat the crop with a fungicide programme. A combination of early treatment and effective chemistry is highly effective in minimising the impact of foliar disease on the crop. Currently, the guidance for fungicide use in the UK is to spray the entire crop at the first visible sign of disease, and not before.

In the UK this was, until recently, commonly a combination of azole and strobilurin fungicides. Due to the structural similarities between strobilurins, they must be used in combination with another fungicide group to decrease the resistance pressure. Before the ban of cyproconazole-containing products in 2021, Escolta was amongst the most popular treatments for fungal diseases in the UK. This product contains cyproconazole which belongs to the azole group of fungicides, and azoxystrobin which is a strobilurin fungicide. Until recently, SDHI (succinate dehydrogenase inhibitor) fungicides were routinely used in many other crops in the UK, but there was no registered SDHI-containing product for UK sugar beet crops. Revystar XE (mefentrifluconazole + fluxapyroxad, BASF) gained registration in 2023 for use on sugar beet, a product which had already previously been registered in other crops. Caligula (fluopyram + prothioconazole, Bayer) has also recently been registered for UK sugar beet. The SDHI component in both of these treatments provides more effective control of cercospora leaf spot compared to azole and strobilurin products.

The next section explores the different major fungicide groups and their modes of action.

1.5.1 Azoles

Azoles are a set of fungicides which are grouped by their mode of action, which is to inhibit the synthesis of ergosterol, an important component in the cell membrane of fungi. Specifically, azole fungicides interfere with the enzyme 14α-demethylase, which is responsible for the demethylation steps in the biosynthetic pathway between lanosterol and ergosterol. Ergosterol is an essential structural component of fungal cell membranes, and its role is similar to that of cholesterol in animal cells.

Azoles (and azole combinations) are the most common fungicide group chosen for treating foliar diseases, and have been for decades (Jørgensen & Heick, 2021). There are more than 25 different azoles which have been developed for use against plant pathogens, and these show less risk of fungicide resistance than strobilurin fungicides, providing longer-term usage with strong effectiveness.

Examples of azole fungicides include propiconazole, tebuconazole and cyproconazole.

1.5.2 Strobilurins

Strobilurins are a set of fungicides which are grouped by their mode of action, which is to inhibit the respiratory chain of fungi at complex III. They are part of the larger group known as Quinone outside inhibitors (Q_OIs). Complex III (also known as the cytochrome bc1 complex) plays a vital role in the production of ATP (adenosine triphosphate), so targeting this enzyme is an effective way to prevent metabolism and growth of fungal cells. The fungicide binds to the Qo site of the cytochrome b subunit, preventing normal electron transfer at this stage.

Strobilurins as a group contain the original natural products, initially found in the fungus *Strobilurus tenacellus*, as well as synthetic products based on the same structure as these natural forms. The synthetic products were developed to improve the activity of the original fungicides, as well as increasing their photostability. Strobilurins tend to be contact fungicides, which means they are absorbed into the leaf cuticle and act locally, but do not get transported within the plant vascular structure.

Natural strobilurins include Strobilurin A and Strobilurin B, synthetic strobilurins include Azoxystrobin, Trifloxystrobin, Kresoxim methyl, and Pyraclostrobin.

1.5.3 SDHIs

SDHI fungicides (succinate dehydrogenase inhibitors) are a group of fungicides which inhibit activity of the enzyme succinate dehydrogenase (also known as complex II in the electron transport chain). This enzyme's role is to catalyse the oxidation of succinate to fumarate, and to transfer electrons from $FADH_2$ to ubiquinone, a crucial step in the production of ATP. The fungicide binds to the ubiquinone-binding site of succinate dehydrogenase, which causes this inhibition. In this way, it has a similar target of activity as strobilurin fungicides, but with strobilurins targeting complex III and SDHIs targeting complex II of the same respiratory chain in the fungal mitochondria.

Succinate dehydrogenase is involved in two separate processes: the mitochondrial electron transport chain, and the citric acid cycle (also known as the Krebs cycle). For this reason, the impact of this fungicide group is two-fold: decreasing ATP production which is detrimental to the energy of the fungal cell, and the accumulation of succinate and decrease in fumarate, which impacts the metabolic processes further.

One of the benefits of the SDHI group of fungicides in comparison to the strobilurin group is that there is more structural diversity in SDHIs than in strobilurins, allowing them to target a large range of fungal diseases without as much risk of developing resistance.

Some examples of SDHI fungicides include carboxin, boscalid, fluopyram, and sedaxane.

1.6 Physiological effects of fungicides on other crops

The relationship between fungicides and physiological improvements in healthy crops has been explored in other crops aside from sugar beet, and various mechanisms have been found to be involved. Many of these mechanisms could apply to sugar beet, therefore a selection of these have been chosen as focus points in this project. An overview of the literature for this topic is explored in this section.

1.6.1 Physiological benefits of fungicides

A study explored the effects of azoxystrobin, pyraclostrobin and boscalid on the physiology and yield of Japanese cucumber (*Cucumis sativus* L.), and found that the fungicide treatments resulted in increased productivity, most evidently so with the pyraclostrobin and boscalid treatments (Amaro et al., 2018).

Another study found that fungicide-treated maize had a better photosynthetic activity while undergoing drought stress, compared with droughted plants which received no fungicide (Sulewska et al., 2019). This relationship has been observed in multiple crops, where there were positive trait responses to fungicides which could not be attributed exclusively to disease control which were more apparent in stress conditions, or that stress-related yield losses were alleviated by fungicides. Another example is a study on the effects of fungicides on tomatoes in droughted conditions, which found that azoxystrobin improved plant water status compared to plants which received no fungicide (Giuliani et al., 2019). Aside from drought stress conditions, this relationship has also been observed in the context of salinity stress. This was demonstrated in a study on salinity-stressed tomato crops, where pyraclostrobin increased chlorophyll content and activity of antioxidant enzymes, reducing the detrimental effects of high salinity on tomato crops (Boari et al, 2019). While this stress-alleviating effect has been extensively demonstrated for strobilurin fungicides, there are also some examples of SDHI fungicides exhibiting a similar effect. One example of this is a study which examined the effects of an SDHI (benzovindiflupyr) on drought-stressed wheat plants, where the SDHI treatment decreased whole-plant transpiration for several days after application, allowing the plants to save water (Kuznetsov et al., 2018). The water-conservative effects in this study resulted in significantly improved yields, which were not due to disease control.

A study on the impact of fluazinam on the physiology of pepper and eggplant crops found that the fungicide promoted an elevated number of mitochondria, an increase in starch granules in the chloroplasts, and other leaf-level physiological benefits (Younes et al., 2020).

These physiological improvements resulted in increased plant length, earlier flowering time, and improved yield traits.

The relationship between fungicide treatments and physiology of tomato plants was investigated by Jacobelis et al. (2023), who found that fungicides increased CO_2 assimilation and production per plant. The same study also found that the fungicide also increased activity of the nitrate reductase enzyme, increased activity of the antioxidant system, and increased the total content of chlorophyll *a* and *b*. Nitrate reductase activity was also observed to increase as a result of strobilurin treatment in Arabidopsis plants, which was suggested to be a major factor in strobilurin-related yield improvements (Van Dingenen et al., 2017).

It's important to note that fungicides from different groups, determined by their mode of action, may interact with crop physiology differently. Additionally, it's useful to observe the effects of fungicides in combinations (e.g. an azole mixed with a strobilurin), as these may have additional benefits to each fungicide applied alone, and combined group fungicides are commonly used in agriculture. An example which demonstrates this is a study which investigated the effects of adding various strobilurin fungicides to an existing azole-only fungicide programme in winter wheat. The experiments revealed that adding strobilurins to the programme resulted in reduced disease levels, delayed flag leaf senescence, and increased yields compared to the azole-only programme (Ruske et al., 2003). Another study which demonstrated the benefit of combining multiple fungicide groups investigated the physiological and yield responses of fungicide-treated winter rapeseed (*Brassica napus* L.). This study found that the application of fungicides had variable effects on crop physiology over the experiment, but that the azole + strobilurin combinations consistently resulted in physiological benefits which increased seed yield (Ijaz & Honermeier, 2012). These effects included reduced lodging and delayed leaf senescence, both of which effectively increased the green area index over the growing period of the crop.

Delayed leaf senescence caused by fungicides has frequently been demonstrated in the literature. One example of this is a study which compared several groups of fungicides on the apparent delay of senescence in wheat, and found that every fungicide in the study had a later senescence than the untreated controls (Berdugo et al., 2012). In this same study, the yields were recorded and all fungicides significantly increased yields compared to the untreated control, and bixafen (an SDHI fungicide) had significantly higher yields than all other fungicides. Another study investigated the effect of mandestrobin (a strobilurin fungicide) on a selection of plants, and found that the fungicide delayed chlorophyll degradation in *Brassica napus* plants (Ido et al., 2020). This study revealed that the mandestrobin treatment resulted in a downregulation in chlorophyll degradation genes and in jasmonate-related genes, while resulting in an upregulation in salicylate-related genes.

This project focuses on fungicides applied to the canopy, typically some months into the growth of the plants, but fungicides in the context of seed treatments have also been shown in the literature to demonstrate improved physiological function in young plants. While fungicides can protect seedlings from soil borne pathogens, they can have differing effects on other physiological activities of the plant. A study on the effect of fungicide seed treatment on spring barley found that some of the azole fungicides (particularly prothioconazole and tebuconazole) resulted in reduced root growth, while the SDHI fungicides (sedaxane and fluxapyroxad) had improved root length (Radzikowska et al., 2020). The authors noted that this effect would be of particular significance in conditions of low water availability, where longer roots would allow the plants to take up more water and reduce drought stress in the early stages of growth. SDHI seed treatments have also been observed to have biostimulant properties in maize seedlings, where sedaxane enhanced root length, area, and forks (Dal Cortivo et al., 2017).

An important concept to consider is that there could be some effect of the fungicides which appears to be additional to disease control, or in the absence of disease, where in fact the fungicide is controlling pre-symptomatic or asymptomatic disease. This scenario would appear as exclusively additional to disease control, but any observed improvements to the plant physiology could be partly due to the fungicide eliminating the negative effects of pathogenic organisms while the plant appears healthy. While it is difficult to determine with accuracy the extent to which physiological benefits of a fungicide may be partly due to early disease control, and how much of the effect is due to further interaction with the fungicide, this relationship can be better understood by comparing with another fungicide treatment which reduces disease without the same physiological benefits. One example of this is a study, which compared the effects of fungicides on spring barley, and found that the protectant fungicide chlorothalonil controlled disease to the same level as the systemic fungicides prothioconazole and pyraclostrobin, but did not result in the same physiological benefits as they did (Bingham et al., 2021). While the chlorothalonil treatment resulted in reduced disease severity and a small increase in mean grain weight, the prothioconazole and pyraclostrobin treatments resulted in both of these changes, as well as increased grain number. The combination of increased grain number and increased mean grain weight resulted in improved overall yield. The difference in the response of these fungicides provides a better understanding of the extent to which improvements to crop yields through fungicide use is due to factors unrelated to early disease control. This concept is further explored in section 3.4.5.

1.6.2 Limited physiological benefits

However, some of the positive effects found in crops treated with fungicides must be considered critically, as some studies have found that the positive effects of fungicides on healthy plants are limited. An example of this is a recent study on the effects of fungicides on healthy soybean plants, which found no improvements to physiology, reproductive development or productivity as a result of fungicide application (Junqueira et al., 2021). This study suggested that much of the research on this topic uses plants which are not healthy as the subject, and that the effects are not observed in healthy plants.

One topic which arises in the literature is that while some benefits may be observed with fungicide application, sometimes these effects are mild, and are not economically viable in cases where disease pressure is low or not present at all.

1.6.3 Detrimental effects of fungicides on physiology

In addition to the examples where physiological benefits can be limited or non-existent, there are some studies which explore the topic of fungicides actually leading to detrimental effects in crop physiology. For example, a study on tobacco plants found that some negative effects were observed when applying Carbendazim, with these effects being more severe with higher doses of the fungicide (García et al., 2001).

Additionally to excessive dosage, premature application of fungicides has also been associated with detrimental effects on physiology and yields. This concept has been demonstrated in a study which applied fungicides to cucumber plants at two early stages (at seed and seedling), and found that the treated plants exhibited reduced stem growth, decreased activity of the phytase enzyme, and disrupted the energetic status and antioxidative system compared to the untreated control (Kafka et al., 2023).

1.7 Research questions

While the physiological effects of fungicides have been explored in a range of crops, there is a knowledge gap for this concept in sugar beet. A selection of topics were chosen as focus points for this project, to determine the effects of a range of fungicides on sugar beet physiology and yields in various conditions. The following research questions and hypotheses were developed after the literature review of this topic:

1. Do certain fungicides increase leaf greenness and other indicators of higher chlorophyll in sugar beet, compared to plants which received no fungicide?

Hypothesis: One or more of the fungicide treatments will result in higher leaf greenness and other indicators of higher chlorophyll content than the non-fungicide control.

2. If leaf greenness is increased by fungicide treatment, does this greenness continue later into the season, as to represent delayed senescence?

Hypothesis: Leaf greenness will be higher than the non-fungicide controls later into the season.

3. Do the positive effects of fungicides occur similarly in disease susceptible varieties and disease resistant varieties?

Hypothesis: Positive effects of fungicides will be observed in both the disease susceptible and disease resistant varieties, improving confidence that effects are not exclusively due to pre-symptomatic disease control.

4. Do sugar beet plants have more resilience against the effects of drought stress if they have been treated with fungicides?

Hypothesis: Sugar beet plants will have more resilience against the effects of drought stress if they have been treated with fungicides.

5. Do fungicides alleviate the negative effects of insufficient nitrogen availability on sugar beet growth and yield?

Hypothesis: The effects of insufficient nitrogen availability will be more severe in plants which received no fungicide than in plants which received a fungicide.

6. Do all fungicide groups result in the same physiological effects, aside from disease control, or do some fungicide groups have different physiological effects to others?

Hypothesis: There will be differences between the physiological and yield effects of some of the fungicide groups.

1.8 Thesis layout

This thesis is formed of three major research areas which have been written in results chapters in paper format. The title and description of each of these following chapters are outlined below.

Chapter 2. The effect of fungicides on sugar beet's response to decreased water availability.

This chapter explored the concept of fungicides improving plant stress tolerance, focusing specifically on low water availability/drought stress. In other crops, certain fungicides have been shown to improve the plant's ability to continue photosynthesising in conditions of low water availability. The results of this study were different to expected based on the literature, with fungicides only improving physiology in well-watered conditions, and showing no significant improvements compared to the non-fungicide control in droughted conditions. The ability of sugar beet to continue photosynthesising even in periods of drought is explored as a likely contributor to these results.

Chapter 3. The effect of fungicides on physiology and yields of a range of varieties of sugar beet.

This chapter explored the effects of a large range of fungicides with contrasting modes of action on two varieties with different disease susceptibility. The purpose was to determine if the positive effects observed in fungicide-treated plants could be exclusively due to pre-symptomatic disease control or if other effects were occurring. If the effects were only significant in the disease susceptible variety, this would have indicated that other effects may not be present. Effects of fungicides were similar in both varieties, despite a larger disease presence in the susceptible variety, which improved confidence that effects were not exclusively due to disease control.

Chapter 4. The effect of fungicides on sugar beet's response to insufficient nitrogen.

This chapter explored the effects of fungicides on sugar beet physiology under varying levels of nitrogen availability. Other crops have been shown to cope better with insufficient nitrogen levels if they have received a fungicide treatment. The purpose of this study was to determine if one of the major reasons that sugar beet appears to have improved physiology under fungicide treatment is that the plants are able to improve their nitrogen uptake and metabolism. In these experiments, the azole + SDHI fungicide appeared to reduce stress responses in the plants and have improved physiological performance, and this fungicide had higher sugar yields at final harvest than the no fungicide control in the field experiment.

Chapter 5. General discussion of the physiological effects of fungicides on sugar beet growth and yields.

This chapter provided a general discussion to draw results from each of the chapters together, link findings mechanistically where possible and explore any recurring themes and conclusions.

Due to the structure of this thesis, references are included at the end of each chapter in which they appear.

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Chapter 2. The effect of fungicides on sugar beet's response to decreased water availability

Abstract

Inadequate water availability is the most limiting factor for crop growth and biomass generation worldwide, and droughted conditions can lead to significant yield loss across a range of crops. Sugar beet is grown in temperate climates, where droughts are usually mild and occur later in the season if they do occur, but such droughts have been shown to reduce sugar beet yields by as much as 30%. Some research has shown that, in other crops, fungicide application has been associated with improved stress tolerance, including drought stress. The main objective of this experiment was to determine if fungicides are associated with improved drought-stress tolerance in sugar beet, allowing the crop to retain its productivity and yield in conditions of lower water availability. The experiment comprised sugar beet plants grown in soil boxes, treated with a range of fungicides under either a standard watering regime or a temporary drought. Physiological measurements including leaf greenness and photosynthesis activity were taken throughout the season and yield data collected at the end of the experiment. Disease pressure was high, particularly of powdery mildew, and this was especially severe in untreated plants towards the latter end of measurements. The Xemium (SDHI) treatment increased several physiological traits and sugar yield compared with the strobilurin treatment. However, this effect was observed only under well-watered conditions. Under droughted conditions, there were no significant differences between fungicide treatments. This experiment was limited in size (three replicate blocks) and was not repeated, so does not determine with certainty whether this relationship does occur in sugar beet at the field scale, but there are several reasons that this relationship might not reflect the current research carried out on other crops. This includes the context that sugar beet is able to continue photosynthesis in droughted conditions, and appears to cope better with recovery after drought than other crops, so they might not experience stress to the same extent as other studied crops when water availability is low. This difference in stress response might be one of the reasons that fungicides did not rescue physiological function as observed in other crop species under drought.

2.1 Introduction

2.1.1 Optimal water availability for sugar beet

Water availability must be adequate for successful crop production, as dry matter accumulation for a given genotype and environment is directly proportional to water use. The optimal rainfall for sugar beet production depends on the temperature and climate, but ranges from approximately 450 mm between April and October in Eastern England to much higher values in the warmer growing areas such as California, USA (Draycott, 2003). Sugar beet is grown in temperate and subtropical climates, where temperatures are cooler than is required for sugar cane, with the leading producers including Russia, the USA, France, and Germany. In the UK, sugar beet is mostly grown on the east side of England, where soil is often more sandy and well-drained than other UK soils, meaning that there can be less available water in the soil, increasing the risk of droughted conditions for crops.

Sugar beet is considered to require moderately high volumes of water for growth, partly due to the long growing season where water requirements must be met throughout. In countries with adequate rainfall, irrigation is not standard practice. However, in semi-arid areas including much of southern Europe, irrigation is required to meet the needs of the sugar beet.

2.1.2 Effects of drought on sugar beet

In sugar beet, insufficient water availability is understood to be the major cause of yield reduction worldwide, causing yield losses as high as 30% in many areas of Europe (Pidgeon et al., 2001). Water stress can affect sugar beet biomass production at all stages, including 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). Due to largely adequate rainfall in the UK, irrigation is not usually an economically viable intervention. However, periods of drought are not uncommon and are an increasing threat due to climate change (Allen et al., 2010). Ober et al. (2010) demonstrated that growing sugar beet in droughted conditions reduces yields and can decrease yield stability, emphasising the need to investigate any interventions which may alleviate the effects of low water availability.

The year following this experiment (2022) was a clear example of the impact of drought, where summer rainfall across the UK was as low as 62% of the 1991-2020 summer average (Met Office, 2022, **Figure 2.1**), with record high temperatures also recorded. During the year of this experiment (2021), weather was generally close to the long-term mean, so experimental conditions were suitable to test the current hypothesis. As the experiment in this chapter was in a polytunnel, watering was controlled and so the conditions would have been suitable even in a year with atypical weather.

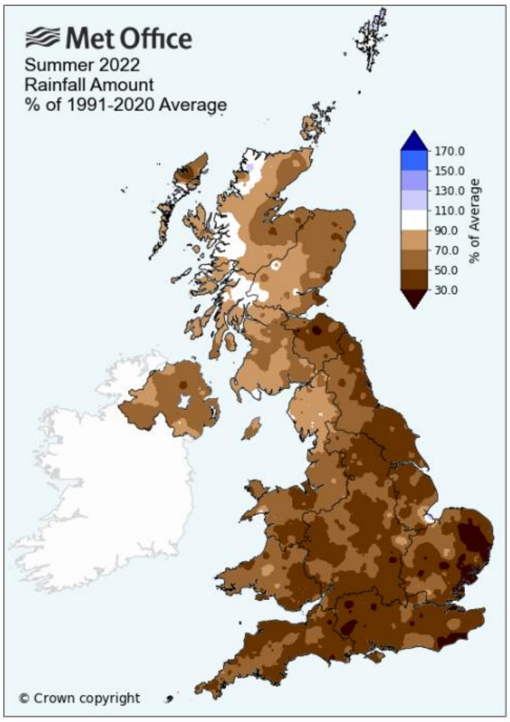

Figure 2.1 Summer 2022 rainfall compared with 1991-2020 average, notably a significantly dry summer compared to expected UK conditions (Met Office).

The direct impacts of drought on sugar beet production should be considered alongside other abiotic stresses that may be affected by climate change and combined with drought. For example, soil salinity increases in droughted soils, which is a yield impacting factor by itself, as well as causing a cumulative yield loss when combined with drought (Bohnert et al., 1995). Both salinity and drought inhibit plant biomass production by reducing photosynthesis, nutrient metabolism, chlorophyll production and respiration among other physiological functions (Tan et al., 2023). Although sugar beet is characteristically tolerant to both drought and salinity compared with many other crops, the impact of each on yield is still substantial (Wedeking et al., 2017). Additionally, periods of drought tend to occur alongside a higher temperature, which has a combined effect on growing conditions.

Inadequate water availability also compounds issues surrounding nutrient uptake, as it reduces nutrient mobility in the soil and plant tissues. Even if nutrient content in the soil is adequate, if water content is too low this will not reach the plant, and if nutrient content in the soil is already low, drought stress will only compound this effect.

For sugar beet canopies, which grow close to the ground compared with many other crops, drought stress can also lead to increased risk of scorching. This is due to wilted leaves sitting flat on the soil, where they are more likely to become damaged by increased heat than if they remained above the soil. This leads to more canopy loss which reduces light interception. Additionally, to recover the canopy area after leaf loss the sugar beet plants have to produce more above-ground biomass which can be at the expense of the root content.

The impact of drought, with and without compounding factors, is clearly an important area of concern for sugar beet production in a changing climate.

2.1.3 Fungicides and stress tolerance

Strobilurins have been associated with improved growth and yield of crops in the absence of disease, including tomatoes and soybeans. Studies on 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 strobilurin used in this experiment was Pyraclostrobin, which appears abundantly in the literature as a treatment with yield-enhancing qualities. Another more recent study also looked at tomatoes but with a different strobilurin, azoxystrobin, which also improved the plant water status under drought conditions (Giuliani et al., 2019). Yield of maize has been shown to increase by up to 5% under treatment by Pyraclostrobin (Nelson and Meinhardt, 2011).

Pyraclostrobin and epoxiconazole have been demonstrated to alleviate some of the negative effects caused by drought stress on different varieties of maize, including effects on yield (Sulewska et al., 2019).

The present experiment was used to compare the response to drought stress between different fungicide treatments in the absence of disease pressure, in order to determine if fungicides can reduce stress-induced yield losses in sugar beet.

Aim

i. To investigate the effects of drought stress on sugar beet in the absence of disease pressure while treated with fungicide containing an SDHI, a strobilurin or left untreated.

Objectives

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

2.2. Materials and methods

2.2.1 Experimental design

Variety

The variety Kortessa was used (from breeder KWS UK Ltd) – with low susceptibility to both powdery mildew and rust compared to others on the BBRO recommended list. Seeds were sown on the 9th March 2021. To closely resemble field conditions, seeds were sown in 3 rows with 30cm row spacing, and approximately 28cm between each of the four plants in each row.

Treatments

1. No fungicide
2. SDHI (Xemium 62.5 g/l, 1 l/ha)
3. Strobilurin (Pyraclostrobin 200 g/l, 1 l/ha)

Plants were treated with fungicides three times, on 11 June, 5 July, and 2 August 2021. The first application was made when the plants were of appropriate growth stage and size, and the following treatments were made after an interval similar to standard practice in field.

The experiment used a split plot design, with watering regime randomised on the main plots and fungicide treatment randomised on the sub-plots with three replicates. The standard watering regime was on a timed irrigation system with drip lines between each row of plants, which watered the soil in the boxes 3 times a week. The droughted watering regime followed the same irrigation system for the beginning of the experiment, but the taps for these specific boxes were turned off from the 9th June 2021, and were turned back on on the 7th July 2021 to begin to return the plants to normal conditions. Between 7th July 2021 and 2nd August 2021, the droughted boxes were included in the irrigation regime 2 days a week, 1 day less than the full watered regime, after which all boxes received the same watering regime. This produced an environment of temporary drought, followed by a recovery and return to standard conditions.

At the beginning of the experiment, volumetric water sensors (ECH$_2$O EC-5, Meter group Inc, Pullman, Washington, USA) were buried at 30cm and used to record the % soil moisture in the boxes. However, as the trial went on the sensors often displayed data which did not reflect the condition of the boxes, so the droughting was instead based on a timing regime instead of a goal droughted %. The disparity seen between the moisture data and the true moisture could have been caused by cracks in the soil caused by extreme droughting, where the sensor was against an air gap instead of being fully submerged in soil.

Layout

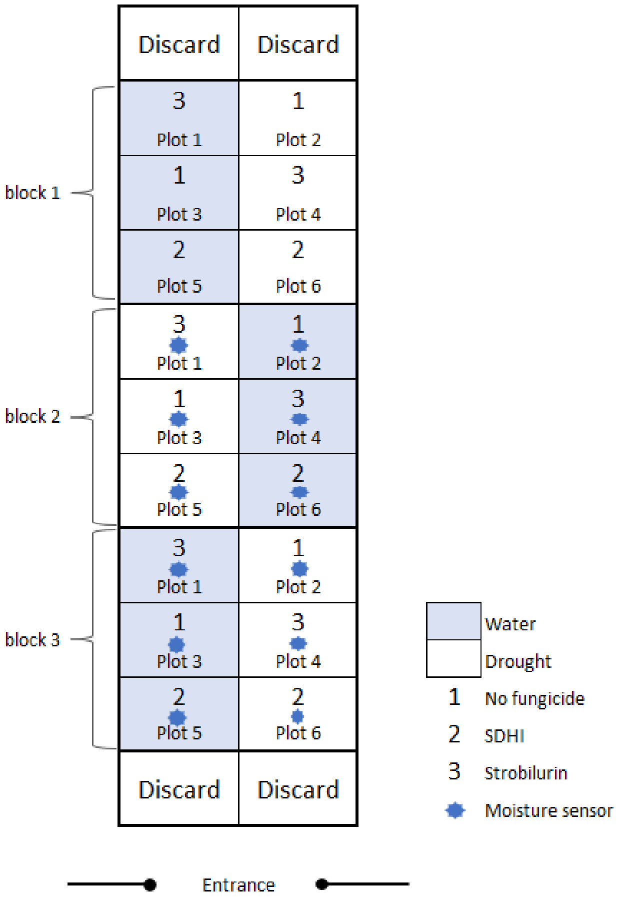

Figure 2.2 Layout of polytunnel experiment using a split plot design with watering randomised on the main plots and fungicide treatment randomised on the sub plots.

Each box contained 12 plants. To ensure successful emergence, seeds were triple sown on 9th March 2021, and seedlings were thinned out to 12 plants per box on 15th April 2021, with similar spacing as in commercial sugar beet fields. Unless stated otherwise, all physiological readings were taken on the two central plants of each box, **Figure 2.3**. The boxes were plastic pallet boxes with a volume of 610L and a depth of 60cm.

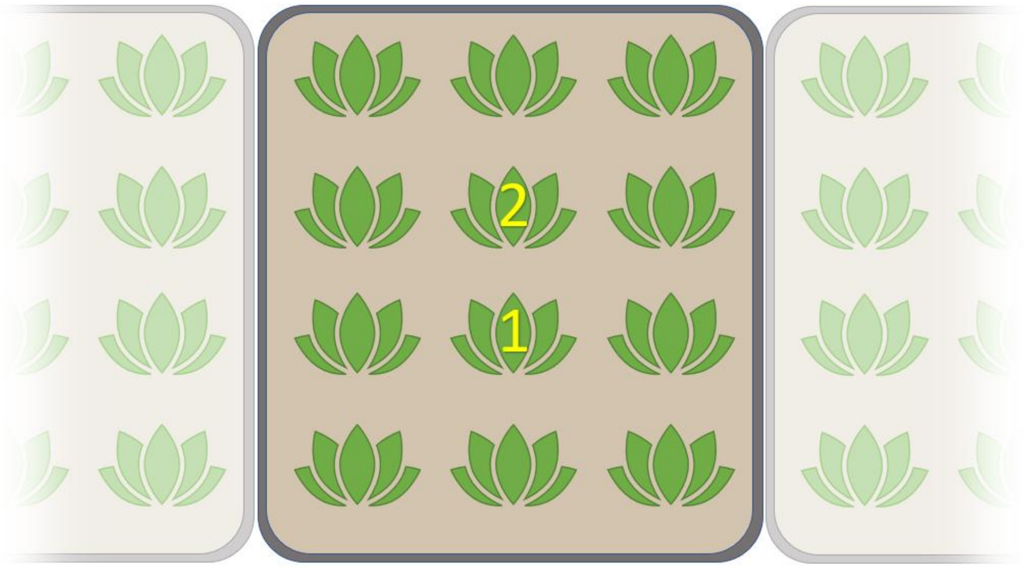

Figure 2.3 Layout of polytunnel boxes, with the two central plants used in physiological data collection highlighted.

2.2.2 Plant measurements

2.2.2.1 Leaf chlorophyll content and Quantum Yield

A SPAD chlorophyll meter (SPAD, Minolta Camera Co., Osaka, Japan) and a Fluorpen (PSI, Drásov, Czech Republic) were used approximately weekly in the polytunnel from 11 June to 22 September to record leaf chlorophyll content (SPAD) and quantum yield (QY). The two newest fully expanded leaves were used from each of the two central sample plants per soil box, 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 used.

2.2.2.2 Spectral reflectance indices

An ASD FieldSpec spectroradiometer (ASD, Malvern Panalytical Ltd., Malvern, UK) was used approximately weekly in the polytunnel from 17 June to 30 September. The two newest fully expanded leaves were used from each of the two central sample plants, for a total of four readings per box. The leaf clip was secured onto the leaf, avoiding large veins or damaged sections, and once the graph on the FieldSpec software had settled and remained unchanged for several seconds, the measurement was recorded. From the outputs of reflectance from wavelengths 350-2000 nm, a selection of published reflectance indices were calculated.

2.2.2.3 Canopy temperature measurements

Temperature of the canopy surface was measured using a handheld thermal camera (C2, FLIR, Wilsonville, Oregon, USA) to give an indication of stress in the plants at the measurement date. Readings were taken on 22 July 2021, a hot day with a maximum temperature of 28°C.

2.2.2.4 Leaf photosynthesis rate and stomatal conductance

An LI-6800 portable photosynthesis system (LI-COR, Lincoln, Nebraska, USA) was used on 29 September 2021 to compare leaf CO_2 assimilation and stomatal conductance between treatments. The newest fully expanded leaf was chosen, and the leaf cuvette was closed onto the leaf until all conditions within the cuvette were showing as stable on the LI-COR. There was one spot measurement taken per plant, on the two central plants of each box. The measurement was taken and later exported from the LI-COR for processing. In the cuvette, the settings were: temperature 20°C, CO_2 400µmol mol^{-1}, PAR 1200 µmol m^{-2} s^{-1}.

2.2.2.5 Harvest measurements

Plants were harvested on the 5 October 2021. From each box, the central two plants were used for growth analysis in the laboratory (leaf weight, petiole weight, root weight and leaf area), while the outer 10 plants were topped (separating the crown from the root at the lowest leaf scar, aiming for a consistent area of exposed crown between each plant), and the roots sent to BBRO for sugar %, root weight, and impurity analysis. Leaf area was measured using a LI3100C leaf area meter (Li-COR Inc., Biosciences, Lincoln, NE, USA). In this year of the experiment, specific leaf weight was calculated using the whole leaf, which included major leaf veins. In the following years, specific leaf weight was calculated using leaf discs, avoiding major leaf veins.

Disease presence was not quantified during this experiment, which reduces confidence in any conclusions about effects which are seemingly separate from disease control. Disease incidence was generally low for the majority of the experiment, however powdery mildew appeared across all plants later in the experiment.

2.2.3 Statistical 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 irrigation on the main plots and fungicide treatment on the sub plots. 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.

2.3. Results

2.3.1 Leaf chlorophyll content

Leaf SPAD was highly variable throughout the season, but using a repeated measures analysis, there was an interaction between fungicide treatment, irrigation treatment, and assessment time (p=0.042, **Figures 2.4a** and **2.4b**). There was also an interaction between irrigation treatment and time (p=0.017), which could be explained by the drought conditions occurring for a temporary section of the data collection period. Under well-watered conditions, the relative chlorophyll content 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 leaf greenness than the SDHI and untreated control. The graphs show well-watered and droughted conditions in **Figures 2.4a** and **2.4b**, respectively.

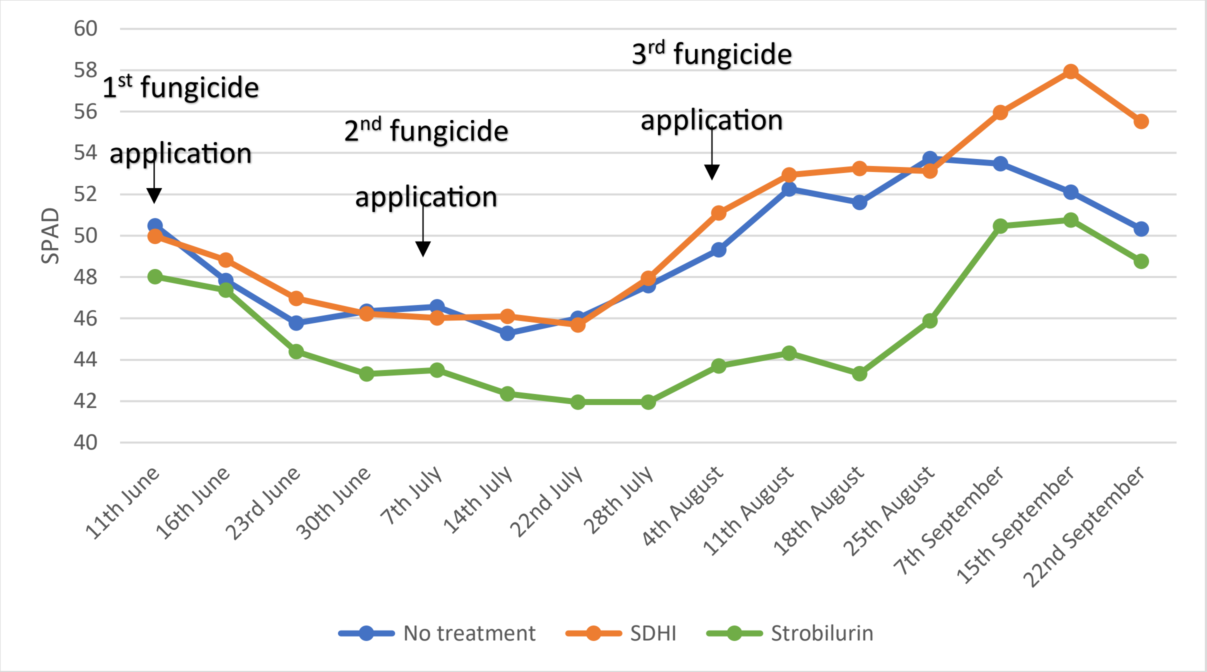

Figure 2.4a Leaf chlorophyll content (SPAD) depending on treatment with either an SDHI, strobilurin, or non-treated control under well-watered conditions. Repeated measures, P = 0.042 for the interaction between fungicide treatment, irrigation treatment, and measurement time.

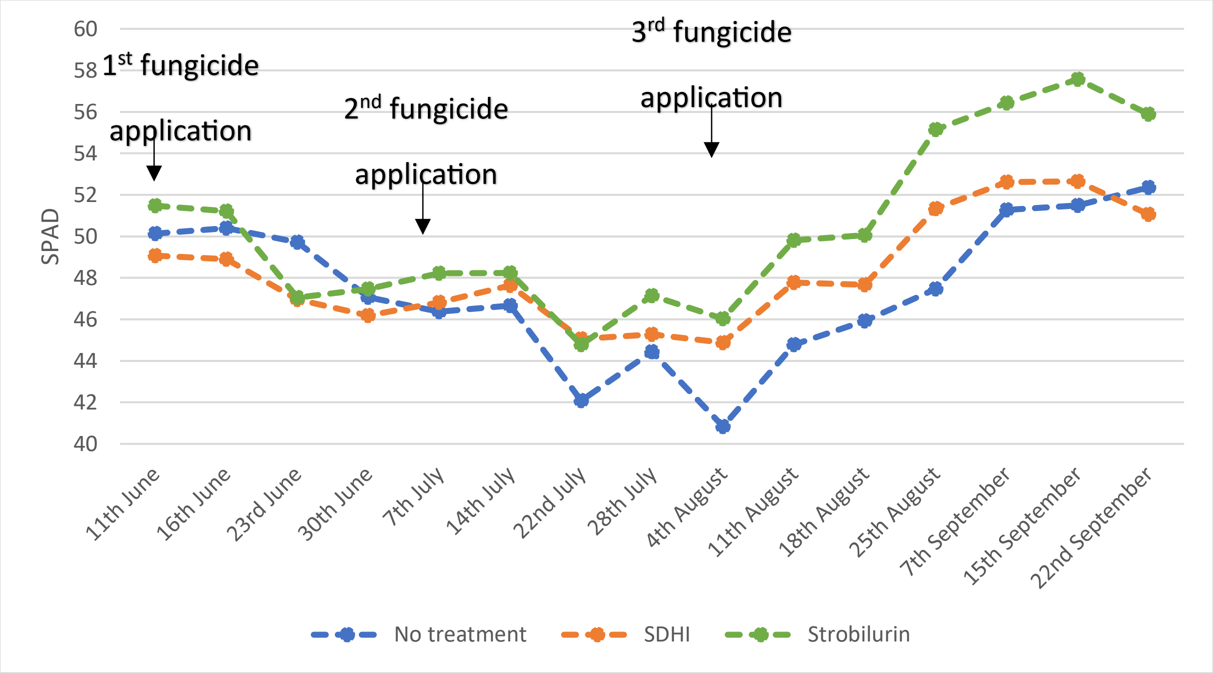

Figure 2.4b Leaf chlorophyll content (SPAD) depending on treatment with either an SDHI, strobilurin or non-treated control, for plants growing in droughted conditions. The irrigation

system was switched off for these boxes between 9th June and 7th July. Between 7th July and 2nd August, these boxes received two thirds of the water compared to the well-watered boxes. After 2nd August, the boxes received the same water as the well-watered boxes.

These results suggest that the strobilurin fungicide might help the plant to stay greener particularly while undergoing drought stress. Alternatively, these results may indicate a difference in leaf thickness to be a combined effect of fungicide and watering regime, but confidence in this is limited from this set of measurements alone.

2.3.2 Leaf chlorophyll fluorescence (Quantum Yield)

Similarly to leaf SPAD, QY was highly variable each week and generally there were no significant differences between treatments. Unfortunately, even with repeated measures analysis, no differences were seen between treatments for QY.

2.3.3 Spectral reflectance indices

Several reflectance indices, including normalised pigment chlorophyll index (NPCI), blue-band modified normalised difference index (mNDblue), ratio analysis of reflectance spectra chlorophyll b (RARSb) and blue green index (BGI), revealed differences between fungicide treatments in some weeks. However, these differences were not consistent and a repeated measures analysis did not reveal significant differences (data not shown). There was often high variability and large standard errors, which could have been due to the small scale of the experiment.

2.3.4 Canopy temperature

Canopy temperature did not differ significantly between fungicide treatments. However, there was a trend for the canopies of droughted plants to be warmer than well-watered plants. These data were collected in the morning while the temperature was low (~9.30 am), when a second set of data was collected around 1.30 pm on the same day, these trends were not observed. There was no significant interaction observed between the drought stress and fungicide treatment (**Figure 2.5**).

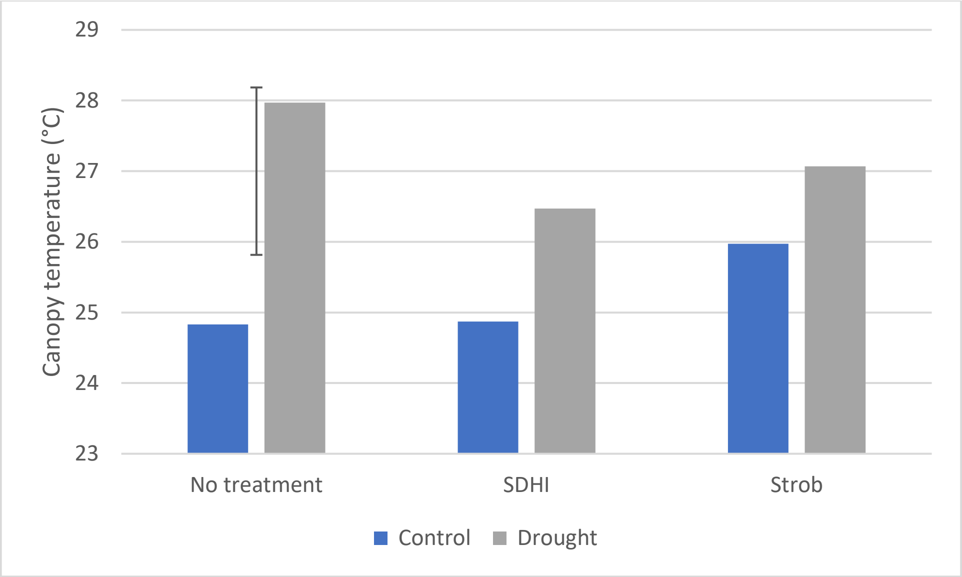

Figure 2.5 Comparison of canopy temperature between droughted and well-watered plants, when treated with an SDHI, a strobilurin, or untreated, no significant difference observed. Measurements were taken on the 22nd July 2021.

2.3.5 Leaf gas-exchange results

The LI-COR data revealed a trend for an interaction between fungicide and irrigation for CO_2 assimilation (p=0.104) and stomatal conductance (p=0.096). In general, the plants performed similarly regardless of fungicide treatment when droughted, but under well-watered conditions, the SDHI and strobilurin treatments had higher CO_2 assimilation rate and stomatal conductance than the control. This is displayed below in **Figures 2.6** and **2.7**. For stomatal conductance, there was also a trend for differences among the fungicide treatments (p=0.086), where SDHI > strobilurin > no fungicide.

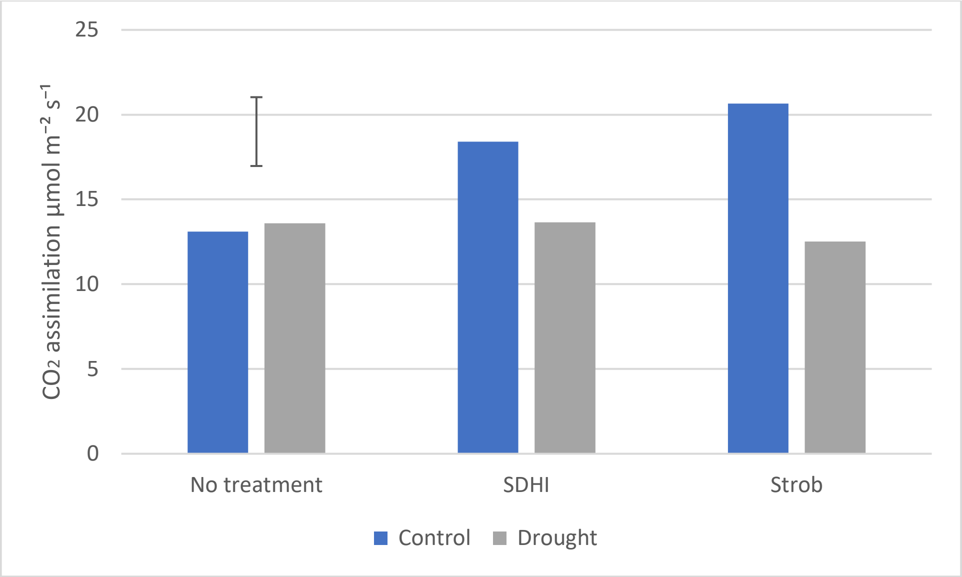

Figure 2.6 Comparison of CO_2 assimilation between treatments depending on watering regime. Measurements were taken on the 29th September 2021. P = 0.104.

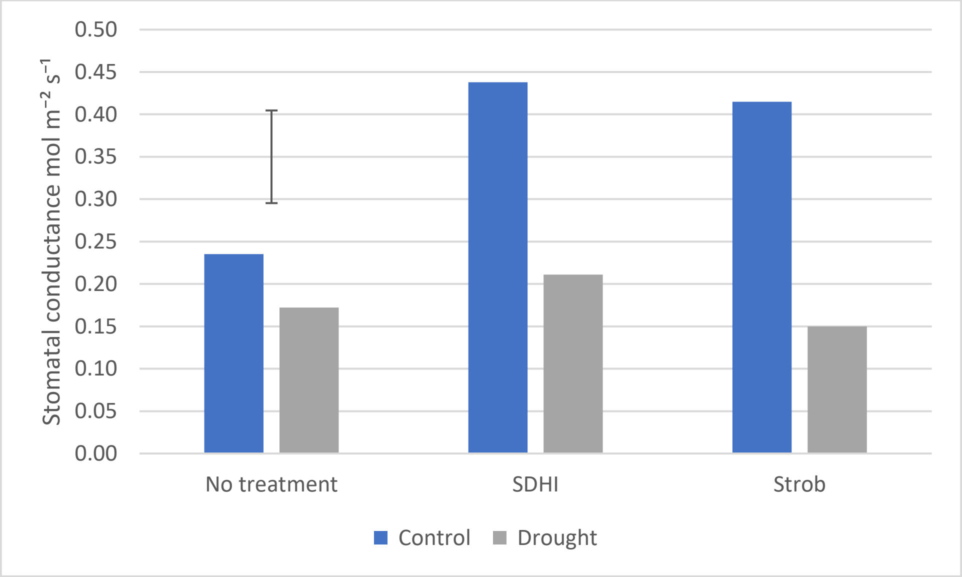

Figure 2.7 Comparison of stomatal conductance between treatments depending on watering regime. Measurements were taken on the 29th September 2021. P = 0.096.

2.3.6 Biomass and sugar yield

Fungicide treatment had no effect on leaf area of droughted plants. However, overall SDHI- and strobilurin-treated plants had much larger leaf area than the untreated plants (p=0.053, **Figure 2.8**). The droughted plants had significantly lower leaf area than the watered plants (p=0.034). There was also a significant interaction between the irrigation and the fungicide treatment (p=0.023), in which the difference in leaf area between droughted and well-watered plants was less detectable in the non-fungicide controls than in the fungicide treated plants.

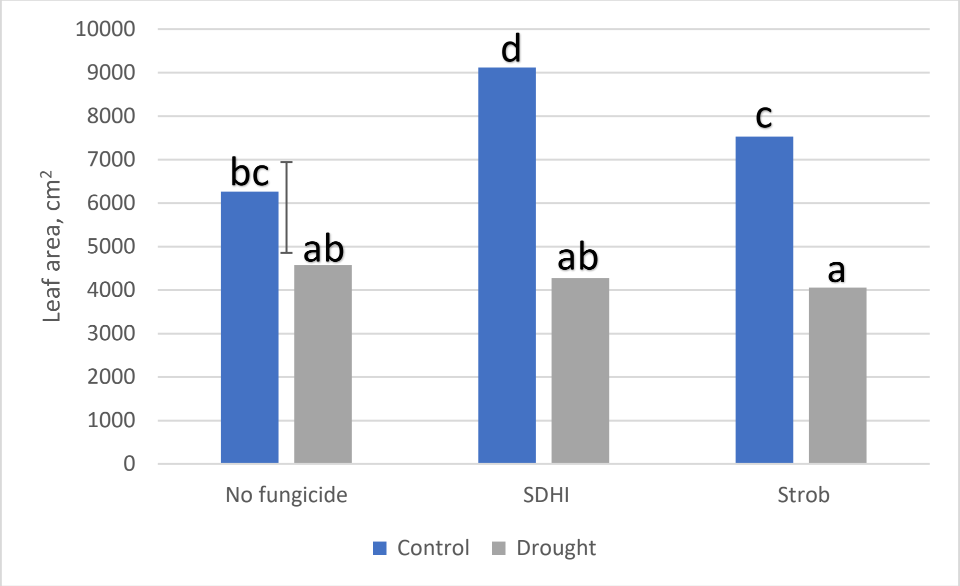

Figure 2.8 Comparison of total leaf area depending on treatment with either an SDHI, strobilurin or untreated control when undergoing either well-watered or droughted conditions, p = 0.023. Duncan's multiple range analysis using only the fungicides regardless of irrigation showed no fungicide (a), strobilurin (ab) and SDHI (b). Letters a-d representing Fisher's protected least significant difference test outputs.

Fungicide treatment had no effect on the total biomass when plants were droughted, however under well-watered conditions, SDHI-treated plants had an increased total biomass compared to the strobilurin treated plants (p=0.034, **Figure 2.9**). Droughted plants had significantly lower total biomass than watered plants (p<0.001).

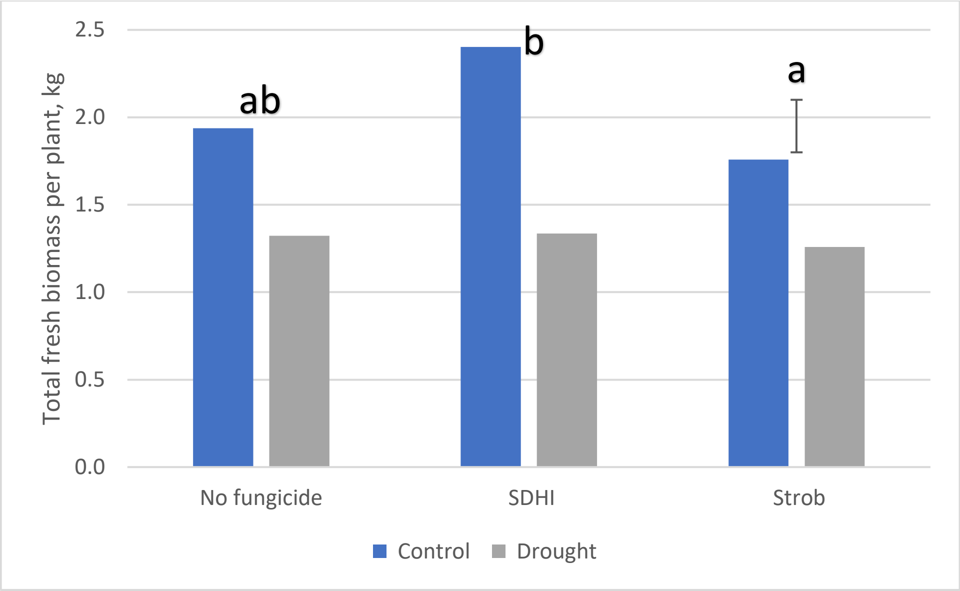

Figure 2.9 Comparison of total fresh biomass per plant between plants treated with an SDHI, a strobilurin or left untreated while being well-watered or droughted. Multiple comparisons using Duncan's multiple range test on the effect of fungicide treatment only (p = 0.034) are displayed, which comprises of both watering regimes.

The dry weights of roots from plants treated with the SDHI were larger than the roots from plants treated with the strobilurin (almost statistically significant, p=0.055, **Figure 2.10.** This figure contains mean dry weights for each fungicide, averaged across both watering treatments). The roots of droughted plants were significantly smaller than those of watered plants (p=0.052), however, there was no significant interaction between fungicide and watering regime for root weight.

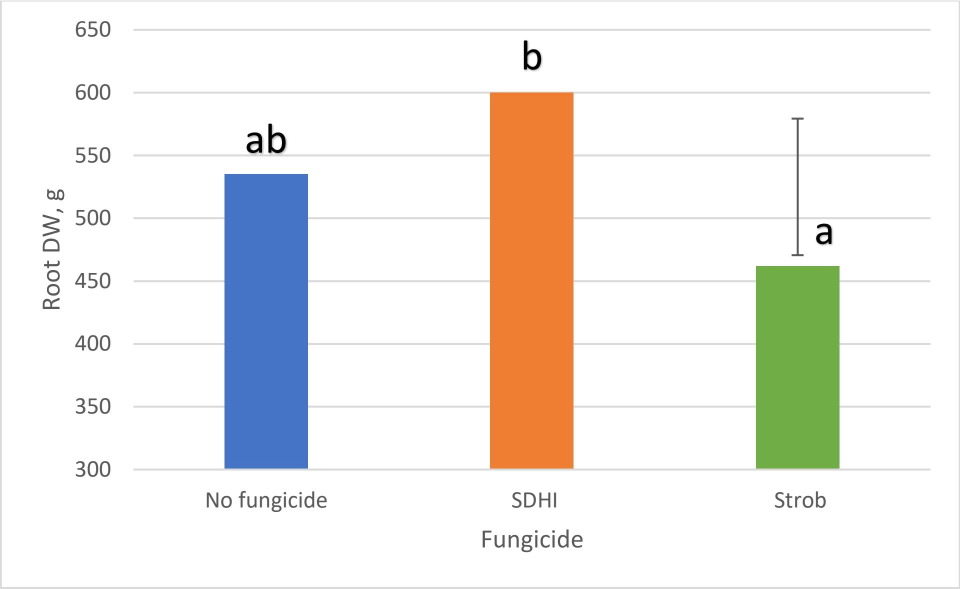

Figure 2.10 Comparison of root dry weight between plants treated with an SDHI, a strobilurin or left untreated, p = 0.055. These dry weights are the means across both water treatments. Sample is of the two central plants in each box.

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 (**Figure 2.11**). The order of the treatments from highest to lowest, however, was still the same as the dry weights in **Figure 2.10**; SDHI > no fungicide > strobilurin. The edge effects in the boxes were large, and this variation may have contributed to the lack of significance for the fungicide treatment. Plants which received standard irrigation had significantly higher total sugar yield compared with droughted conditions (p=0.005).

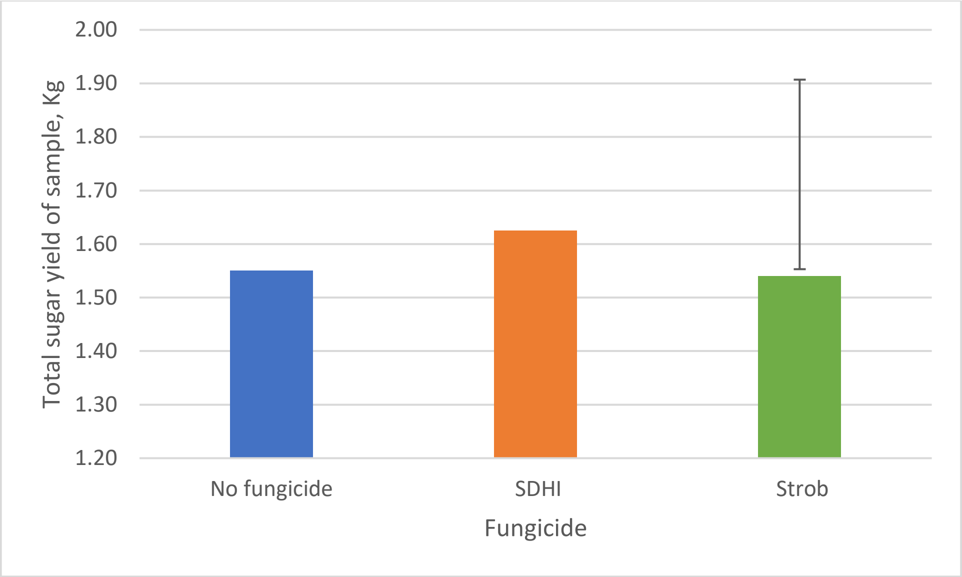

Figure 2.11 Comparison of total sugar yield from the outer 10 plants of each box, between fungicide treatments. No significant difference observed.

2.4. Discussion

The polytunnel experiment overall showed clear impacts of drought on sugar beet physiology and yield. For most of the physiological traits measured in this experiment, well-watered plants performed significantly better than droughted plants, and final yields reflected this difference. This was demonstrated by the observation that droughted plants had a significantly lower root dry weight than well-watered plants.

In terms of the effects of fungicides, this experiment showed the SDHI treatment to be most favourable in terms of root weight, and this was reflected in several physiological traits, including leaf area. The strobilurin treatment mostly outperformed the non-fungicide control for physiological traits, but yields for strobilurin-treated plants were not significantly higher than other treatments. For sugar yield, there were no significant differences between fungicide treatments, although the order of highest to lowest was still SDHI, no fungicide, then strobilurin, matching the order for root dry weight. Towards the end of the experiment, there was a moderate incidence of powdery mildew across all plants, however this was not quantified in this experiment. While there is very limited confidence in scaling up from such a small sample size, for the sake of comparison using the 10 outer plants from each box, the equivalent sugar yields work out as approximately 15.5t/ha for no fungicide control, 16.25t/ha for SDHI and 15.4t/ha for strobilurin. This is assuming that there are 100,000 plants per hectare, and that plants contain the sugar yields found in Figure 2.11.

2.4.1 Physiological basis of fungicide-treatment effects

The leaf SPAD provides an estimate of chlorophyll content, so it was surprising to see that in well-watered conditions, the strobilurin treatment consistently had lower values than the non-fungicide control, when strobilurin treatments have been shown to increase chlorophyll content in other crops (Julião et al., 2020).

Additionally, leaf SPAD for the strobilurin treatment was increased under drought. This was not the case for the no-fungicide and the SDHI, where SPAD values decreased under droughted conditions. It is possible that SPAD values can become positively skewed when plants are wilting, or leaf expansion is restricted under drought due to leaf chlorophyll being concentrated into a smaller area, as SPAD essentially represents chlorophyll density. It might therefore be considered that the high SPAD observed with droughted strobilurin-treated plants was due to a concentration effect, and it would have been helpful to have compared the area of each leaf alongside the SPAD measurement, to determine if the effect could have been due to thicker leaves with lower leaf area.

Canopy temperature results appeared to have some trends for differences between treatments but these were not statistically significant. More frequent measurements may have provided a more useful insight into the relationship between fungicide treatment and canopy temperature. With a higher canopy temperature being associated with a more stressed canopy which is unable to maintain transpiration to sustain a cooler leaf surface in hot weather, there is an indication that plants without fungicide had reduced ability to continue these processes hence reduced photosynthetic capacity than those treated with fungicides. Therefore the fungicides may have been allowing the canopy to maintain open stomata and transpiration. Without statistically significant differences, confidence in this effect is limited, and further experiments with more frequent measurements are required.

2.4.2 Harvest results

2.4.2.1 Physiological basis of the fungicide * irrigation interactions

The results in this experiment show that fungicides appeared to increase leaf area compared to plants which receive no fungicide under well-watered conditions, however the experiment was unable to detect this same relationship between treatments when plants were grown in droughted conditions. Two perspectives are required when exploring the further information provided by multiple comparisons analysis; comparing only the fungicide treatments, and comparing how each treatment responds to watering vs drought.

For the first perspective, multiple comparisons showed that the strobilurin treatment was not significantly different from the other two treatments, and that the only clear difference was between the SDHI treatment and the non-fungicide control. With regard to the treatment interaction, the well-watered SDHI treatment and the well-watered strobilurin treatment increased compared to the droughted treatments, but the well-watered no-fungicide control did not. Thus, both fungicides provided a boost to leaf area compared against no fungicide but only in well-watered conditions. However, the strobilurin may not have been as beneficial as the SDHI in this instance.

This interaction was also observed for SDHI compared to the no-fungicide control for total biomass although not for root weight nor total sugar. It may also suggest that leaf area is not a suitable predictor of the other harvest measurements, and that while strobilurin may well increase leaf area, this may not translate to improved root weight. A possible reason for this trend is that the effects on leaf area and subsequent light interception did not have enough time to translate to yield accumulation, and that an effect may have been seen if the leaf area differences persisted for longer before harvest. Another possibility is that the impact of lower canopy area may have been less severe in the polytunnel due to the polythene covering of the polytunnel leading to a more uniform and diffused light source than in field conditions. A larger trial, potentially field based, would provide a better understanding of the nature of this relationship.

2.4.3 Effects of fungicides on other droughted crops

It is possible that the drought in the present experiment was not severe enough to observe the expected responses to the fungicides. A study investigating the impact of pyraclostrobin and epoxiconazole on maize varieties under drought stress found that leaf photosynthetic efficiency during drought stress was higher in plants treated with fungicides compared to those left untreated (Sulewska et al., 2019). The response differed between the two varieties they investigated, with one variety displaying a larger improvement to photosynthetic rate after treatment than the other variety, but the other variety showing a better regeneration response after treatment. The impact of drought stress on maize yields was demonstrated to be highly dependent on the phase of growth in which the stress is induced, including the highly productive stage of flowering. The authors emphasised the severity of the impact of drought stress on maize compared with other crops. Another study demonstrated in a meta-analysis that reducing the water availability by approximately 40% led to 39.3% yield reduction in maize, compared with 20.6% in wheat (Daryanto et al., 2016).

A recent study in soybeans showed that application of a fungicide reduced the impact of drought stress, specifically displaying an improvement in nitrogen metabolism, CO_2 fixation, and chlorophyll retention compared to droughted plants without fungicide applied (Kanungo et al., 2021). The fungicide used in this publication was a combination of Epoxiconazole and Pyraclostrobin, the latter of which was used singly in this current study. The data in this chapter about photosynthetic activity in sugar beet shows that Pyraclostrobin (the strobilurin) did increase CO_2 assimilation under well-watered conditions, like in the soybeans in the experiments of Kanungo et al. (2021).

2.4.4 Sink limited crop, limiting yield benefits

In this experiment, fungicides did not improve sugar yields under droughted conditions even though yield improvement has been observed in other crops. One possible explanation for this is that the crop's production may be more limited by the expansion of the root (sink) under drought than by source compared to other crops. This is relevant because the impact of fungicides on photosynthetic capacity under drought stress may not translate to improved yield. Many of the physiological effects of fungicides affect source traits, leaf growth, photosynthetic activity, stomatal conductance etc., rather than improving the root expansion under droughted conditions. This limitation is not only relevant for investigating fungicides' ability to reduce yield loss under drought, but for any factor which is expected to improve drought-stress tolerance through improvement of canopy productivity rather than root expansion.

This may provide a reasonable suggestion for the lack of interactions observed in drought experiments in sugar beet compared with other crops; that most of the growth stages in sugar beet appear not to be source limited, so factors that impact canopy cover have less of an interaction with yields compared to crops which are heavily limited by light interception (Hoffmann, 2019). For example, a treatment which increases leaf area might significantly remedy a droughted crop which is limited by light interception, but if a sugar beet's root expansion has been limited by drought, then a difference in leaf area is not going to be enough to change the yield. This perspective could also explain why a reduction in leaf area and other source traits did affect yields in well-watered conditions, as they were likely not sink-limited in the same way that droughted plants were. This suggestion is further validated by the publication discussed earlier about maize's response to drought depending heavily on the stage of growth, with more severe impact at more productive stages (Sulewska et al., 2019), which could be a reason that factors which improve photosynthetic function under drought conditions may have more impact on yield in other crops compared to sugar beet. It would therefore be reasonable to assume that fungicides which exclusively improve canopy productivity rather than root growth will be more likely to improve yields of drought stressed plants which are source limited rather than sink limited.

2.4.5 Drought recovery in sugar beet

Another possible explanation for the fungicides not reducing yield loss under droughted conditions is that sugar beet is known to recover well from drought compared to other crops (Wedeking et al., 2017). So, under mild drought while the fungicides might be having some interaction with the plant's response to drought, the plants were able to recover from the drought stress even in the no-fungicide plants.

Recent research on water-use efficiency in sugar beet showed that the crop is still able to photosynthesise effectively during periods of drought, even if wilting is evident (Barratt et al., 2021). Barratt et al. (2021) showed that sugar beet leaves had a higher number of smaller stomata, compared to spinach which had fewer but larger stomata, and that the smaller stomata could be the reason that sugar beet was quicker to respond to changes in light. The study showed that under drought stress, sugar beet stomata opened more slowly and were faster to close compared to well-watered plants, but that there was still a stomatal response to changes in light. This meant that they continued to use available water and photosynthesise effectively, which suggests that wilting is not necessarily detrimental to the photosynthetic apparatus in the leaves. In periods of severe and long-term drought, this could be a disadvantage where the plant does not conserve water effectively. However, with the nature of droughts being short-term in temperate climates where sugar beet is cultivated (Jaggard et al., 1998), this continued activity is an advantage. The understanding that sugar beet responds differently to drought compared with other crops provides a useful perspective for the current study; that the drought response which is seen to be alleviated by fungicides in other crops may be different to the response seen in sugar beet.

Sugar beet’s ability to cope with - and recover well from - drought and salinity is often attributed to the crop’s ancestry as a maritime plant (Ribeiro et al., 2016). This could be one of the main reasons that sugar beet’s response to drought is different to many other crops, and therefore the effect of fungicides on drought response was not able to reflect that seen in other crops in this study.

2.4.6 Drought is not the only stress

It’s important to consider that although the current study was not able to show fungicides alleviating drought stress in sugar beet, it does not mean that this relationship does not exist, nor that this would not be seen for any other type of stress. Soil salinity was not recorded in this experiment, so conclusions cannot be drawn about the effect of drought with salinity considered. This experiment used polytunnel boxes, and drought was short-term, however, on a field scale and after periods of long-term drought, salinity would increase in the soil (Bohnert et al., 1995). This warrants the consideration that fungicides might improve sugar beet’s response to drought, but that the polytunnel set-up did not produce a drought representative of what would occur in the field. Physiological responses to several different types of stress can be similar, because restriction of water availability is a common factor in not only drought but also in salinity and freezing stress. This means it’s likely that some responses would be seen in many different stress sources, however, the specific responses cannot be assumed to be common across all. This experiment only investigated drought, so further research could investigate the effects of fungicides on a wider range of abiotic stress factors.

2.4.7 Stress at the cellular level

Considering these differences between sugar beet and other crops’ responses to drought stress, it is valuable to explore the responses at the cellular and hormonal level, to further identify where these differences may take place. Several studies have explored the mediating ability of triazole applications to stressed plants, and the themes which arise are an increase in cytokinins, a rise in abscisic acid (ABA), and a decrease in ethylene compared with stressed plants without triazole application (Desta & Amare, 2021). Triazoles are a wide group of chemicals, some of which are used as fungicides and others as plant growth regulators, so it is important to consider that while there may be similarities in the cell interactions for the triazole applications in these reported studies, they may not represent the entire triazole group.

2.4.7.1 Relevance of ABA

ABA is a phytohormone responsible for several physiological responses, including promoting adaptive responses to stress. Specifically, ABA is involved with stomatal closure under droughted conditions, protecting the plant from excessive water loss (Tuteja, 2007). ABA's activity is induced by stress within the plant, and research shows that ABA deficient mutant plants fail to protect themselves from stress, wilting readily and dying if stress continues, even when the mutants appeared normal under non-stressed conditions (Swamy & Smith, 1999). This emphasises the importance of ABA regulation in drought-stressed plants, and provides some understanding of the improved stress tolerance of plants treated with fungicides, which can be shown to increase ABA accumulation.

Azoles and ABA

A study published in 1990 explored the response of bean (*Phaseolus vulgaris*) plants to uniconazole applications, which is an azole product primarily used as a plant growth regulator, and found that it increased the accumulation of ABA (Mackay et al., 1990).

Strobilurins and ABA

A study investigating the effect of strobilurins on ABA content in grapevine plants found that ABA is increased in the plants after strobilurin application, but returned to untreated levels within three days (Diaz-Espejo et al., 2012). Although this ABA increase was transient, the study found that treated plants produced significantly higher yields. The authors noted that the stomata may have additionally been responding directly to the changes in ATP concentrations caused by strobilurin application, as a result of temporary blocking of the cytochrome-bc1 complex of the electron transfer chain. The mechanism of guard cell osmoregulation is known to be proportional to ATP concentration, and is one of the mechanisms involved in stomatal responses. Therefore, the temporary inhibition of ATP synthesis by strobilurin application could be responsible for some of the observed changes in stomatal responses, at least temporarily. This study only found these results under nondroughted field conditions, so while its comparison to the current study is limited, it is still an important result to consider in the topic of fungicide-phytohormone interactions.

SDHIs and ABA

SDHIs effects on ABA (and other phytohormones) are less extensively studied than other fungicide groups due to the more recent introduction to the market of this group. Due to the similar mode of action of SDHIs to strobilurins, it would be useful to investigate the similarities between the two in the context of phytohormone effects.

Further research would be useful to determine the response of ABA levels and subsequent stomatal activity in sugar beet plants treated with fungicide, considering droughted and well-watered plants.

2.4.7.2 Cytokinins in a stress response

In the context of drought-stress responses, cytokinin acts as an antagonist to ABA. In absence of drought conditions, cytokinin signalling suppresses a stress response. However, in droughted conditions cytokinin signalling is reduced, increasing the plant's sensitivity to ABA and mediating the stress response (Huang et al., 2018). A study which investigated cytokinin deficient *Arabidopsis* found that it was more tolerant to drought stress compared to wild type, being able to survive adverse conditions (Nguyen et al., 2016), displaying that this plant hormone could be an area of focus for improving drought tolerance of crops.

Azoles and cytokinins

Perhaps counterintuitively, studies on the impact of triazole compounds on phytohormones actually show that the chemical leads to an increase in cytokinin synthesis (Rabert et al., 2014). This may lead one to assume that these triazoles are therefore actually supressing the useful stress response, which contradicts the results which are seen under these treatments, where triazoles seem to improve the stress response. With this in mind, it is important to differentiate between stress-tolerance by improved survival, and stress-tolerance by continued activity even in adverse conditions. Phytohormones which suppress physiological activity to conserve resources will be helpful for improving survival rates in prolonged or severe stress, but with a cost of reduced growth and yields. On the other hand, phytohormones which promote continued activity even in periods of stress will be more beneficial in conditions of short term or mild stress, where it is not necessary to forfeit yield in the name of survival.

Strobilurins and cytokinins

A study on Kresoxim-methyl, a strobilurin fungicide, found that application of the fungicide increased both ABA and cytokinin levels (Grossmann et al., 1999). The study also demonstrated that application of the fungicide resulted in lower ethylene levels, by inhibiting the production of 1-aminocyclopropane-1-carboxylic acid (ACC), which is a precursor for ethylene. These effects on hormonal balance resulted in darker green leaves, delayed leaf senescence, and reduced water consumption. Ethylene has been shown to be involved in the degradation of cytokinins in the context of stress responses, so lower levels of ethylene result in higher levels of cytokinins. The positive effect of strobilurins on cytokinin activity is therefore at least two-fold, where application directly increases cytokinin signalling, as well as reducing its degradation.

SDHIs and cytokinins

The interaction between SDHIs and cytokinins is less extensively researched than in other fungicide groups, as this group was introduced to the market more recently than azoles and strobilurins. Due to the similar physiological responses to both SDHI and strobilurin treatments, it would be valuable to determine if these similarities also occur at the phytohormone level too.

Having a higher cytokinin level in periods of drought can be associated with improved yields compared with lower cytokinin, through delayed drought-induced senescence of leaves (Rivero et al., 2007), and with continued photosynthesis even during drought (Rivero et al., 2009), which would normally be supressed by an ABA-induced stress response. This relationship is seen in a range of different crops, as outlined in the extensive review of cytokinin response to stress by Cortleven et al. (2018). This has relevance especially in the context of growing areas which tend to have short-term rather than severe and long-term drought, as continued plant productivity can be an advantage in these conditions. Under long-term severe drought, it’s important for the plant to conserve water as much as possible, however in temperate climates droughts tend to be short-lived and a less conservative stress response will be more advantageous.

This poses the question – is sugar beet's apparent high tolerance to drought and subsequent lack of fungicide impact on activity at least in part due to high cytokinin activity compared with other crops? A study on the development of sugar beet leaves and related hormones found that cytokinin concentration was not only high in young leaves, but remained high even in the more mature leaves (Kudoyarova et al., 2018). It is relevant to mention here that cytokinins are understood to reduce the inhibitory effects of glucose on plant physiology (Moore et al., 2003), and that high cytokinin content can prevent senescence caused by high concentration of glucose in leaves (Veselov et al., 2017). This points to the likely assumption that the higher cytokinin found in mature leaves of sugar beet is sufficient to protect them from glucose-induced senescence, and may be one of the explanations for sugar beet's apparent stress tolerance.

A recent study aimed to determine the effects of applying synthetic cytokinins to drought-stressed sugar beet, but was unable to demonstrate a clear improvement in the plants physiology in these conditions (Vomáčka & Pospíšilová, 2003). Looking at the relationship in the absence of drought, focusing only on senescence as a whole, another study was able to show that application of cytokinins to sugar beet plants resulted in higher chlorophyll content, and delayed degradation of nuclei and nuclear DNA, during the senescence process compared with untreated plants (Topchii et al., 2009). The studies may provide support for the results in the current experiment, where some fungicides were observed to result in some improved trait measurements in well-watered conditions but not under droughted conditions. If one of the major effects of fungicides on plant physiology is due to an increase in cytokinin activity, then this effect may be reduced if the plant has already increased its cytokinin activity (i.e. in periods of drought). This relationship is complex, and if a treatment (such as a fungicide) is shown to affect a particular hormone, it must be considered that the treatment is likely also interacting with other hormones which interact with one another. For example, a treatment which affects both ABA and cytokinin activity could have a similar physiological outcome to a treatment which affects one of them, which leads to a knock-on effect on the other.

2.4.7.3 The importance of ethylene

Ethylene is a phytohormone with a range of activities within plants, including fruit ripening, plant growth regulation, and most relevantly to this study – stress response. The activity of ethylene is complex, and can lead to both resistance and susceptibility to stress conditions, depending entirely on the context of the stress, and which hormones it is interacting with (De Vleesschauwer et al., 2010). During stressed conditions, ethylene interacts with other phytohormones to control plant activity and growth as a survival mechanism, in a controlled balance between growing in spite of stress or prioritising resource conservation. When conditions are favourable, ethylene production tends to remain low (except for some specific developmental processes), however, in unfavourable or stressed conditions, ethylene production is increased.

Ethylene and ABA interact in several plant processes, mostly acting antagonistically with each other. For example, seed germination, lateral root emergence, and seedling development are all positively regulated by ethylene and negatively regulated by ABA. In the context of stress, while ethylene can be shown to cause resource-conserving functions such as stomatal closure (Desikan et al., 2006), it is understood that both ethylene and ABA are present, and that ethylene diminishes ABA's conservative action (Tanaka et al., 2005). This demonstrates ethylene's antagonistic activity against ABA, in the context of drought stress.

Ethylene also interacts with the cytokinin signalling pathway, and appears to be capable of suppressing cytokinin activity (Grossmann et al., 1999). This indicates that an intervention which reduces ethylene activity could have a knock-on effect where cytokinin activity is kept higher than controls, allowing the plant to continue photosynthetic activity even during periods of stress.

Reviewing the various interactions between fungicides and hormones which are associated with stress responses and senescence provides a useful perspective to the data observed in the current study. While the current study did not record activity of hormones, some of the resulting effects seen in other studies are comparable to the effects observed in this study. It is therefore possible to consider that the effects of fungicides on plant hormones may be similar in sugar beet to other crops, although the subsequent effect of the activity of these hormones may have differed. Further research into the effects of fungicides on stress responses at the hormone level would provide a better understanding of this relationship.

2.4.8 Limitations

Many other crops demonstrated more resilience to drought when treated with fungicides in the absence of disease compared with untreated (Kanungo et al., 2021), whereas this experiment did not reveal this same relationship. The two fungicide treatments and control did not differ under drought, and it was only under well-watered conditions that the fungicide treatments showed some improved trait responses. In the polytunnel, leaf gas exchange was only measured on one date, on which there was an indication of higher leaf CO_2 assimilation rate in both fungicide treatments compared with the no fungicide control, although this was not statistically significant ($p = 0.104$). It is possible that effects could have changed throughout the year, and a single date might not be representative. A further limitation of this experiment was that after the moisture sensors were no longer providing accurate measurements, irrigation scheduling was based on timings rather than calculated estimated soil moisture deficits, which could have provided a more controlled treatment.

Due to this experiment taking place in a polytunnel with irrigation rather than rainfall, and this irrigation being controlled by time rather than recording the equivalent rainfall in mm, this did not allow for potential soil moisture deficit to be calculated.

The experiment was small, with only 3 replicate blocks, which could have contributed to the difficulty in determining significant differences in the trial. A larger experiment with larger boxes, more plants per box, and 4 blocks instead of 3 would be a useful avenue for future research to gain a clearer insight into the relationship. Additionally, as disease was not quantified in this experiment, it is harder to separate conclusions about physiological benefits without the possible effect of disease control. All field experiments after this experiment had quantified disease measurements.

2.4.9 Conclusions

This study demonstrated that fungicides can improve many physiological traits, such as relative chlorophyll content, stomatal conductance, CO_2 assimilation, and leaf area in well-watered conditions. However, under droughted conditions, this study did not find an effect of fungicide treatment on most physiological traits. Leaf temperature of droughted plants appeared to be lower for the fungicide-treated plants than the no-fungicide control, suggesting that the plants were more able to maintain transpiration and keep the leaf cooler. This could be indicative of a change in the plants stress response, where instead of closing stomata the plant continues activity. Further research into the hormone response of sugar beet plants to drought stress under various fungicide treatments would improve the understanding of this relationship.

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Chapter 3. The effect of fungicides on physiology and yields of a range of varieties of sugar beet.

Abstract

Sugar beet (*Beta vulgaris* subsp. *vulgaris*), commercially grown for its production of sugar, is susceptible to a range of foliar 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 phytotonic 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. The focus of this study was to observe physiological changes as a result of fungicide treatment in field experiments over two years, in order to identify which changes may be related to improved sugar yields. During the first year (2021), fungicide treatments including an azole, an SDHI, and an azole + strobilurin combination were higher than healthy untreated plants for physiological processes like CO_2 assimilation and stomatal conductance, and reflectance indices indicative of chlorophyll content. In terms of harvest root weight data, the top yielding treatments were the azole + strobilurin combination > the strobilurin > the SDHI. 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 weight (leaf thickness). During the second year (2022), treatment effects were less clear, and a severe drought affected some physiological traits from July onwards. However, consistent with the previous year, the azole + strobilurin combination had improved physiological function and higher root yields than the plants which received no fungicide.

3.1. Fungicide + Variety experiment introduction

3.1.1 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 improvement is 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 responses were taking place to improve canopy persistence and radiation-use efficiency. Studies on other crops have also shown similar relationships, including potatoes (MacDonald et al., 2007) and wheat (Ishikawa et al., 2012). Effects of improved stress tolerance are explored in chapter 2 of this thesis, and effects of improved nutrient use efficiency are explored in chapter 4.

3.1.2 Stay-green effect

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 prolonged (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 according to 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.

In winter wheat (*Triticum aestivum* L.), strobilurin treatments have consistently been shown to delay senescence of the flag leaf (Ruske et al., 2003) (Gooding et al., 2000), resulting in a stay-green effect. Delayed senescence in the flag leaf of wheat in particular is important, as yield formation is heavily impacted by flag leaf photosynthesis. The concept to consider here is that any crop in which yield is source limited or co-limited by source and sink will benefit more significantly from delayed senescence, compared to those which are predominantly sink limited. Much of the research surrounding stay-green effects of fungicides focuses on strobilurins and azoles, but there is evidence of delayed senescence of wheat with SDHI treatments (Berdugo et al., 2012).

3.1.3 Increased CO_2 assimilation

The relationship between fungicide application and CO_2 assimilation has been explored in other crops. A study compared several physiological traits between tomato (*Solanum lycopersicum* L.) plants treated with a range of fungicides, and found that fungicides increased CO_2 assimilation (Jacobelis et al., 2023). This relationship has also been demonstrated in soybean (*Glycine max*), where pyraclostrobin was found to increase carbon assimilation in a field experiment (Joshi-Paneri et al., 2023).

3.1.4 Improved water-use efficiency (WUE)

The relationship between fungicides and improved water-use efficiency has been studied in other crops. A study found that pyraclostrobin improved the growth (controlled experiment) and yields (field experiment) of wheat plants grown in water deficit conditions, compared to those which received no fungicide (Inagaki et al, 2009). The study suggested that the fungicide treatment resulted in delayed water uptake through the roots, which postponed the dehydration of the soil and redistributed water uptake towards the grain filling period, resulting in a slight increase of grain yield in some wheat genotypes.

A similar relationship has been observed in tomato plants, where a strobilurin treatment resulted in improved total and marketable yields under water shortage conditions, compared to both untreated and non-strobilurin treated plants.

3.1.5 Pre-symptomatic disease control

A possibility to consider when observing increased growth and yield in seemingly healthy crops treated with fungicides is that the response could be partly due to the control of pre-symptomatic disease. An example of this situation 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 the symptoms had not yet become visible.

3.1.6 Aims and objectives

Currently, the relationship between fungicides and expression of physiology traits in healthy plants has been explored in other crops. However, research on this topic in sugar beet is limited. Understanding the specific physiological responses to fungicides in sugar beet will help inform choices about fungicide usage, combined with disease control abilities. Specifically, if benefits are seen more in a particular group of fungicides in certain conditions, then this could be useful for targeting fungicides in a changing climate. Additionally, if particular fungicides have a more beneficial effect earlier or later in the season, this can help to inform timing of treatment application. The present experiments aimed to determine which physiological traits are affected by fungicide applications in the absence of disease, and to quantify their associations with sugar beet yield.

Overall aim

To investigate the effects of a range of fungicides on sugar beet physiology and resulting sugar yields.

Objectives

Compare plants treated with either Revysol (an **azole**), Xemium (an **SDHI**), Revysol + Xemium (an **azole + SDHI combination**), Cyproconazole + Trifloxystrobin (an **azole + strobilurin combination**), Pyraclostrobin (a **strobilurin**), Difenoconazole + Azoxystrobin (a second **azole + strobilurin combination**), or left untreated in two varieties in field experiments in two years.

a. Compare physiological traits such as canopy cover %, canopy greenness and a range of canopy reflectance indices across fungicide treatments in two varieties with differing disease susceptibility.
b. Compare associations between physiological traits and biomass production and sugar yields.

3.2. Materials and methods

3.2.1 Experimental design

2021

Two varieties were chosen for the field experiment in 2021, with the purpose of providing some insight into the extent to which disease control or phytotonic effects (or pre-symptomatic disease control) were responsible for the increase in yields. If the two varieties with differing disease susceptibility responded differently, it would indicate a larger proportion of improved yields being due to early disease control. The two varieties used were:

- Kortessa (KWS), low susceptibility to foliar diseases

- Advena (KWS), higher susceptibility to foliar diseases

Field information for the 2021 crop is as follows:

- Latitude and longitude of experiment: 52.835875, -1.252628
- SNS N Index: 16kgN/ha, SNS Index 1
- Pre-sowing soil nutrient analysis: P:3, K:2+, Mg:4, pH:7.0
- Fertiliser: 348 kg/ha 34.5% Nitram (120kg/ha N)
- Herbicides were applied as needed to control weeds

Historic weather data (from Met Office – Sutton Bonington station) for 2021:

Year	**Month**	**Max temp (tmax degC)**	**Rain (mm)**
2021	January	5.7	114.6
2021	February	8.4	36.6
2021	March	11.2	28.4
2021	April	11.9	0.6
2021	May	15	87.6
2021	June	20.6	23.2
2021	July	23.7	66.6
2021	August	20.5	37.2
2021	September	21.3	45
2021	October	15.7	85
2021	November	10.9	14
2021	December	8.8	51.4

Treatments

0. No fungicide
1. Azole (Revysol 112.5 g/l, 1.5 l/ha)
2. SDHI (Xemium 62.5 g/l, 1 l/ha)
3. Azole + SDHI (Revysol 100 g/l + Xemium 47 g/l, 1 l/ha)
4. Azole + Strobilurin (Cyproconazole 160 g/l + Trifloxystrobin 375 g/l, 0.35 l/ha)
5. Strobilurin (Pyraclostrobin 200 g/l, 1 l/ha)

The fungicide-treated plots were treated twice, on the 3rd August and the 7th September 2021.

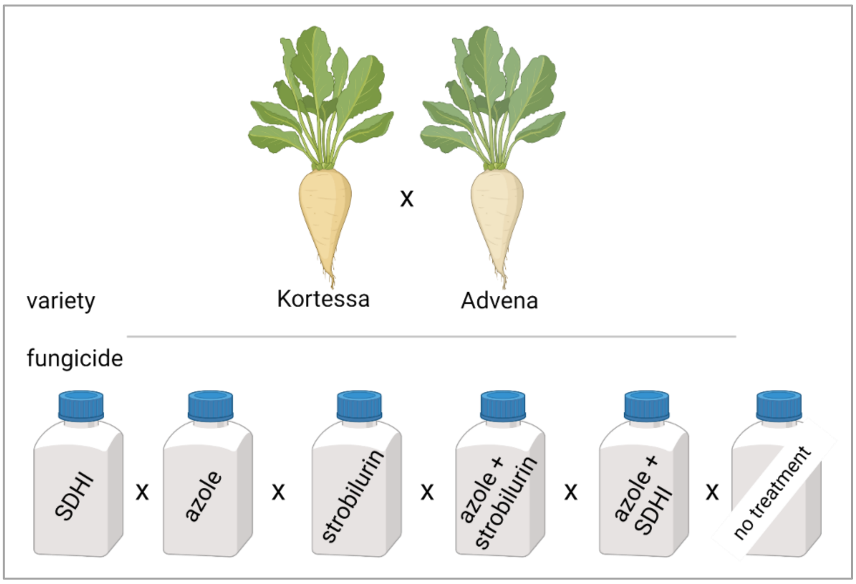
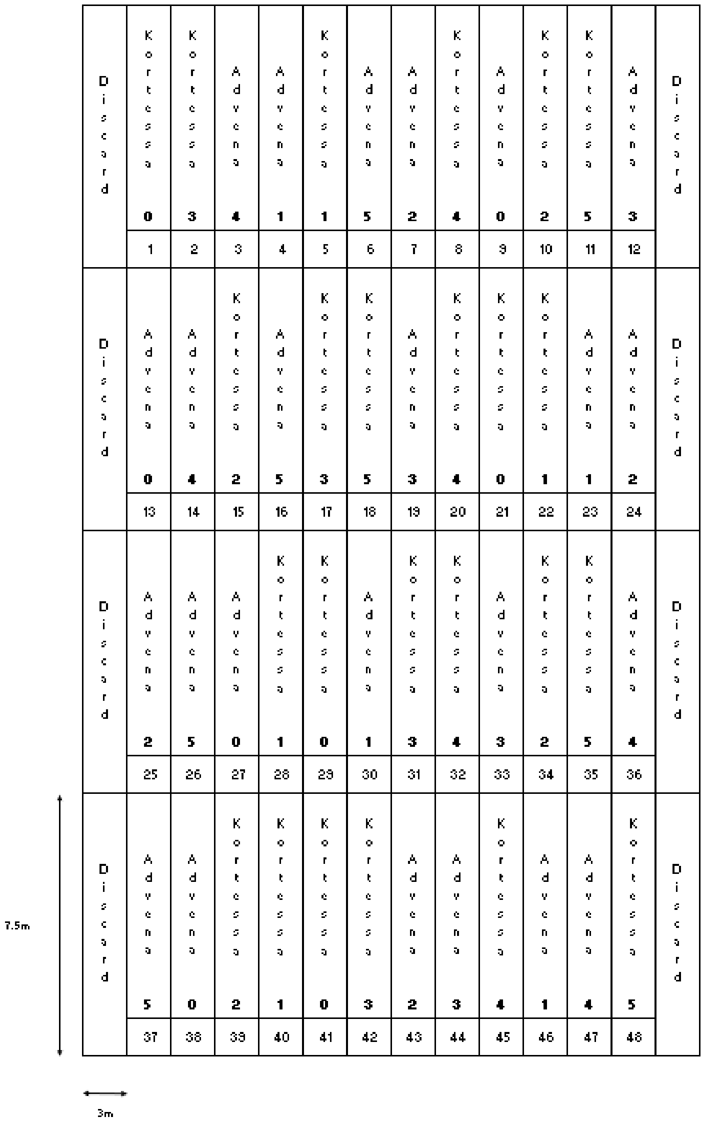


Figure 3.1 Overview of the fungicide treatments and varieties used in the field experiment in 2021.

Experimental design

The experiment consisted of a factorial design with four replicate blocks, each fully randomised with each fungicide and variety combination, as shown in the trial layout below in **Figure 3.2**. Plots were sized 3m * 7.5m, and the row width was 50cm. Plots were drilled on 30th March 2021.



Discard	Kortessa 0	Kortessa 3	Advena 4	Advena 1	Kortessa 1	Advena 5	Advena 2	Kortessa 4	Advena 0	Kortessa 2	Kortessa 5	Advena 3	Discard
	1	2	3	4	5	6	7	8	9	10	11	12	
Discard	Advena 0	Advena 4	Kortessa 2	Advena 5	Kortessa 3	Kortessa 5	Advena 3	Kortessa 4	Kortessa 0	Kortessa 1	Advena 1	Advena 2	Discard
	13	14	15	16	17	18	19	20	21	22	23	24	
Discard	Advena 2	Advena 5	Advena 0	Kortessa 1	Kortessa 0	Advena 1	Kortessa 3	Kortessa 4	Advena 3	Kortessa 2	Kortessa 5	Advena 4	Discard
	25	26	27	28	29	30	31	32	33	34	35	36	
Discard	Advena 5	Advena 0	Kortessa 2	Kortessa 1	Kortessa 0	Kortessa 3	Advena 2	Advena 3	Kortessa 4	Advena 1	Advena 4	Kortessa 5	Discard
	37	38	39	40	41	42	43	44	45	46	47	48	

Figure 3.2 Layout of the field experiment 2021. Fungicide treatments labelled 0-5; 0 = no fungicide, 1 = Azole, 2 = SDHI, 3 = Azole + SDHI, 4 = Azole + Strobilurin, 5 = Strobilurin.

2022

For the 2022 year of the experiment, the same two varieties were included, as well as two additional varieties/ variety combinations.

- 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 were the focus of my data collection. Where differences were seen between all four varieties with differing disease susceptibility, these results are included and explored. However, in the interest of consistent comparison between years, many of the results are further broken down to compare only Kortessa and Advena.

The trial was drilled on 29th March 2022. The fungicide-treated plots were treated three times, with the same fungicides each time, on 31st July, 5th September, and 12th October 2022.

Field information for the 2022 crop is as follows:

- Latitude and longitude of experiment: 52.838073, -1.253536
- SNS N Index: 65kgN/ha, SNS Index 1
- Pre-sowing soil nutrient Index: P:4, K:2+, Mg:4, pH:7.3
- Fertiliser: 116 kg/ha 34.5% Nitram (40kg/ha N)
- Herbicides were applied as needed to control weeds

Historic weather data (from Met Office – Sutton Bonington station) for 2022:

Year	Month	Max temp (tmax degC)	Rain (mm)
2022	January	8.2	24.4
2022	February	10.6	72.8
2022	March	12.2	34.6
2022	April	14	16.2
2022	May	18.2	48.4
2022	June	20.8	32.2
2022	July	24.6	11.6
2022	August	24.8	18
2022	September	19.2	51.2
2022	October	16.9	87.2
2022	November	12	96.8
2022	December	7	42

Treatments

0. No fungicide
1. Azole (Revysol 112.5 g/l, 1.5 l/ha)
2. SDHI (Xemium 62.5 g/l, 1 l/ha)
3. Azole + SDHI (Revysol 100 g/l + Xemium 47 g/l, 1 l/ha)
4. Azole + Strobilurin **A** (Cyproconazole 160 g/l + Trifloxystrobin 375 g/l, 0.35 l/ha)
5. Azole + Strobilurin **B** (Difenoconazole 125 g/l + Azoxystrobin 125 g/l, 1 l/ha)

Treatment 4 (azole + strobilurin **A**) is Escolta, a treatment frequently used on sugar beet crops, but due to a ban on cyproconazole this fungicide is no longer in use. Due to this loss of chemistry, a second azole + strobilurin combination was included (Treatment 5) to calibrate the popular treatment against a newer one within the same fungicide group.

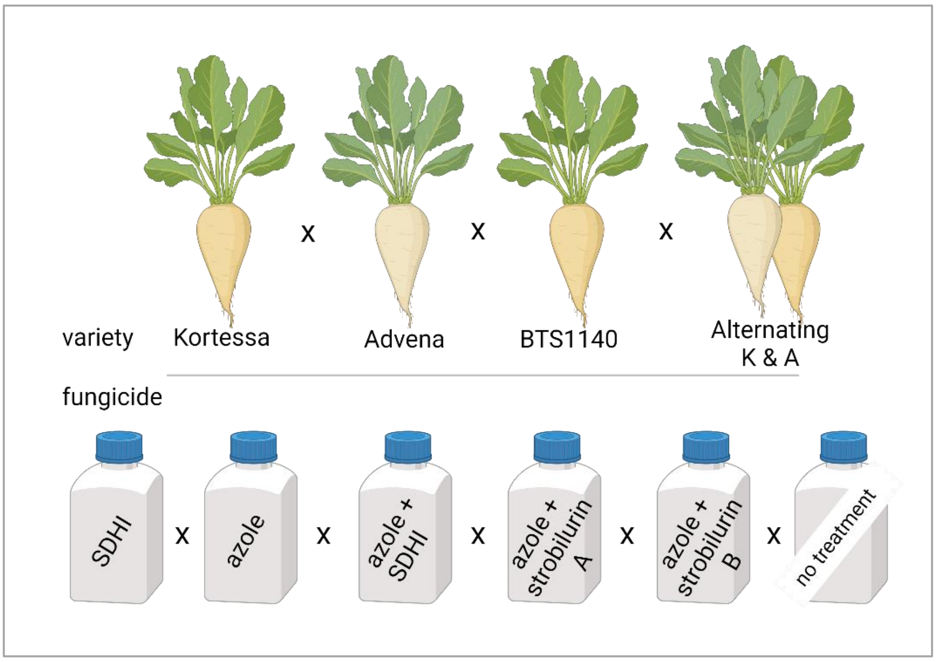
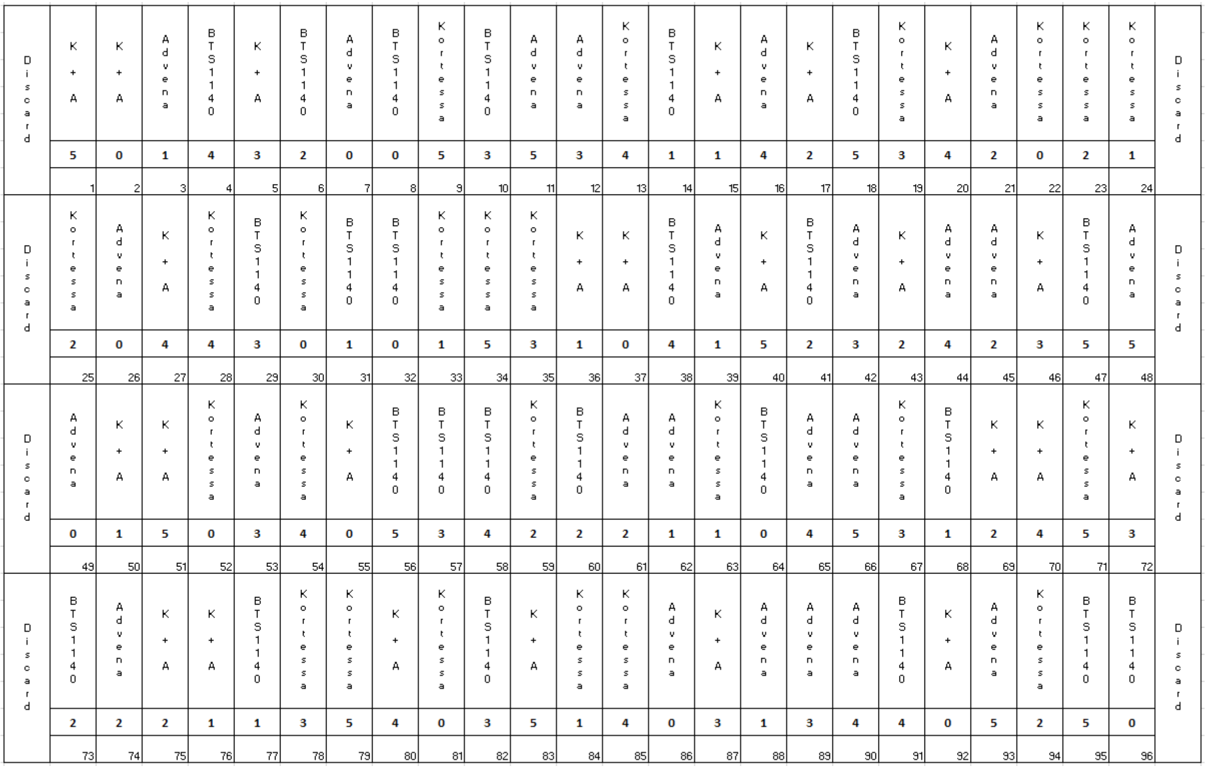


Figure 3.3 Overview of the treatments used in the field experiment 2022.

2022 Layout



Discard	K + A	K + A	Advena	BTS1140	K + A	BTS1140	Advena	BTS1140	Kortessa	BTS1140	Advena	Advena	Kortessa	BTS1140	K + A	Advena	K + A	BTS1140	Kortessa	K + A	Advena	Kortessa	Kortessa	Kortessa	Discard
	5	**0**	**1**	**4**	**3**	**2**	**0**	**0**	**5**	**3**	**5**	**3**	**4**	**1**	**1**	**4**	**2**	**5**	**3**	**4**	**2**	**0**	**2**	**1**	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Discard	Kortessa	Advena	K + A	Kortessa	BTS1140	Kortessa	BTS1140	BTS1140	Kortessa	Kortessa	Kortessa	K + A	K + A	BTS1140	Advena	K + A	BTS1140	Advena	K + A	Advena	Advena	K + A	BTS1140	Advena	Discard
	2	**0**	**4**	**4**	**3**	**0**	**1**	**0**	**1**	**5**	**3**	**1**	**0**	**4**	**1**	**5**	**2**	**3**	**2**	**4**	**2**	**3**	**5**	**5**	
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	
Discard	Advena	K + A	K + A	Kortessa	Advena	Kortessa	K + A	BTS1140	BTS1140	BTS1140	Kortessa	BTS1140	Advena	Advena	Kortessa	BTS1140	Advena	Advena	Kortessa	BTS1140	K + A	K + A	Kortessa	K + A	Discard
	0	**1**	**5**	**0**	**3**	**4**	**0**	**5**	**3**	**4**	**2**	**2**	**2**	**1**	**1**	**0**	**4**	**5**	**3**	**1**	**2**	**4**	**5**	**3**	
	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	
Discard	BTS1140	Advena	K + A	K + A	BTS1140	Kortessa	Kortessa	K + A	Kortessa	BTS1140	K + A	Kortessa	Kortessa	Advena	K + A	Advena	Advena	Advena	BTS1140	K + A	Advena	Kortessa	BTS1140	BTS1140	Discard
	2	**2**	**2**	**1**	**1**	**3**	**5**	**4**	**0**	**3**	**5**	**1**	**4**	**0**	**3**	**1**	**3**	**4**	**4**	**0**	**5**	**2**	**5**	**0**	
	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	

Figure 3.4 Layout of the field experiment 2022. Fungicide treatments labelled 0-5; 0 = no fungicide, 1 = Azole, 2 = SDHI, 3 = Azole + SDHI, 4 = Azole + Strobilurin A, 5 = Azole + Strobilurin B.

3.2.2 Experiment measurements

The following subsections detail data collection methods, which were the same across both years unless otherwise specified.

3.2.2.1 Leaf chlorophyll content (SPAD)

A SPAD chlorophyll meter (SPAD, Minolta Camera Co., Osaka, Japan) was used approximately once per two weeks to record relative leaf chlorophyll content (SPAD). Four plants from within the central rows of each plot were randomly selected, and the newest fully expanded leaf was used.

3.2.2.2 Spectral reflectance indices

An ASD FieldSpec (ASD, Malvern Panalytical Ltd., Malvern, UK) was used to take measurements throughout the season. Four plants from within the central rows of each plot were randomly selected, and the newest fully expanded leaf was used. The leaf clip was secured onto the leaf, avoiding large veins or damaged sections, and once the graph on the

FieldSpec software had settled and remained unchanged for several seconds, the measurement was recorded. From the outputs of reflectance from wavelengths 350-2000nm, a selection of reflectance indices was calculated.

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

3.2.2.3 Canopy cover photographs

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 47 255). If the lighting made these thresholds ineffective at selecting the green area, they were adjusted to suit the whole selection of photographs.

3.2.2.4 Disease scoring

During 2021, disease scoring was only carried out once, However, in 2022, disease scoring was carried out frequently to quantify the presence of common diseases such as rust and Cercospora leaf spot. A % of leaf area showing disease symptoms across the plot was estimated by walking through each plot and observing three central rows.

In the 2021 experiment, disease scoring was recorded during the week commencing 15th November, and in the 2022 experiment it was carried out from the 11th July until the 24th November.

3.2.2.5 Harvest measurements

Plots were initially harvested by removing 10 plants from each plot by hand for laboratory processing. For 2021 this was on 9th December, and for 2022 on 29th November. In each year, the remaining plants in each plot were then removed using a beet harvester and sent for analysis at the beet processing 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 laboratory, more detailed partitioning measurements were taken such as 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 calculate further traits such as specific leaf weight (calculated as g per cm^2 area of leaf, as an indication of the thickness of leaves).

3.2.2.6 Data analysis

An analysis of variance (ANOVA) for the factorial fully randomised block design was carried out using Genstat 19th edition (VSN International, Hemel Hempstead, UK), assuming variety and fungicide were fixed effects, and including replicates as a random effect in the analysis. Where significant differences were reported in the ANOVA, multiple comparisons were calculated using Duncan's multiple range test, 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 at P=0.05 as error bars.

3.3. Results

3.3.1 Leaf chlorophyll content

A repeated measures ANOVA of the 2021 leaf SPAD data revealed that the azole + strobilurin and the azole + SDHI treatments consistently had a higher leaf SPAD value than the azole or the strobilurin treatments (p=0.001, **Figure 3.5**). The azole + strobilurin treatment also had higher spad than the no fungicide treatment. There was no difference in SPAD values between the two varieties, and there was no interaction between the effects of variety and fungicide on leaf SPAD, indicating that the fungicide effects were similar for both varieties.

Table 3.1 Duncan's multiple range test results from a multiple comparisons analysis of SPAD values in the 2021 season. These values are averaged across all dates and both varieties.

Treatment		**Mean**	**Duncan's multiple range test**
Azole + strobilurin	🟦	57.01	c
Azole + SDHI	🟩	56.4	bc
SDHI	🟧	55.45	abc

No fungicide	⬛	55.19	ab
Strobilurin	🟦	54.12	a
Azole	🟨	53.98	a

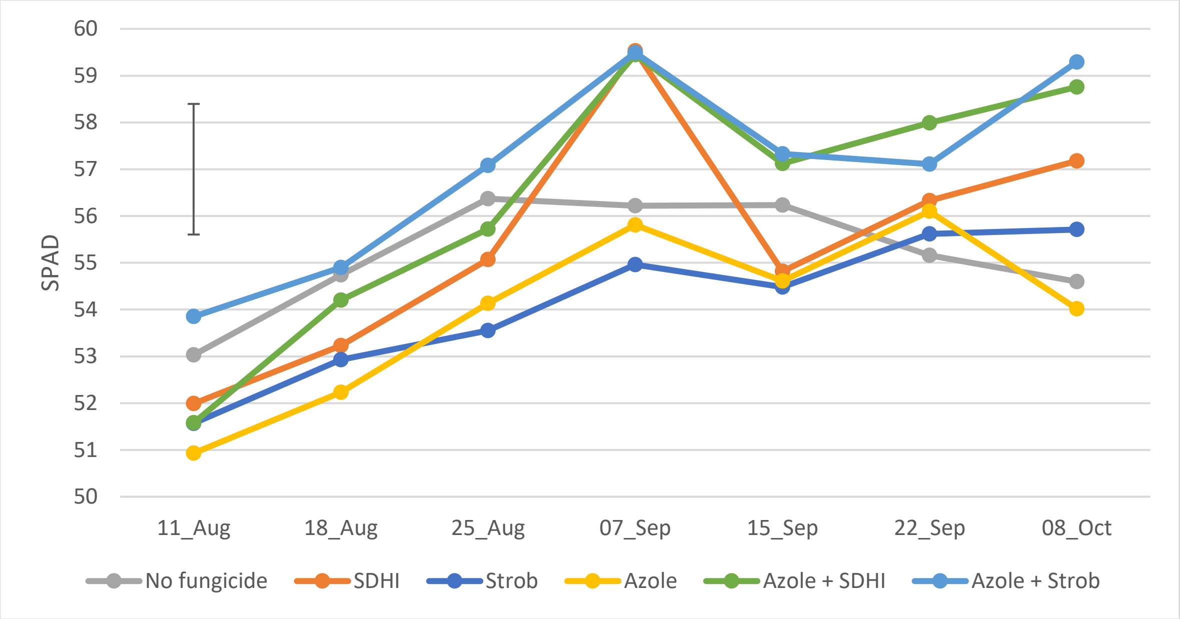


*Figure 3.5 Repeated measures analysis comparing leaf SPAD between treatments in 2021. P = 0.001 for differences between fungicide treatments. Error bar is LSD for time * fungicide repeated measures analysis.*

A repeated measures ANOVA of the 2022 leaf SPAD data, including all varieties in the analysis, showed that for all dates after the first fungicide treatment, the azole + strobilurin A treatment had higher SPAD than the SDHI, azole +strobilurin B, and no fungicide (**Figure 3.6**, p = 0.054). The BTS1140 variety had lower SPAD than the other 3 varieties (p < 0.001).

Table 3.2 Duncan's multiple range test results from a multiple comparisons analysis of SPAD values in the 2022 season These values are averaged across all dates and all varieties.

Treatment		**Mean**	**Duncan's multiple range test**
Azole + strobilurin A	🟦	64.18	b
Azole + SDHI	🟩	63.59	ab
Azole	🟨	63.18	ab
No fungicide	⬛	62.86	a

Azole + strobilurin B	⬛	62.71	a
SDHI	🟧	62.67	a

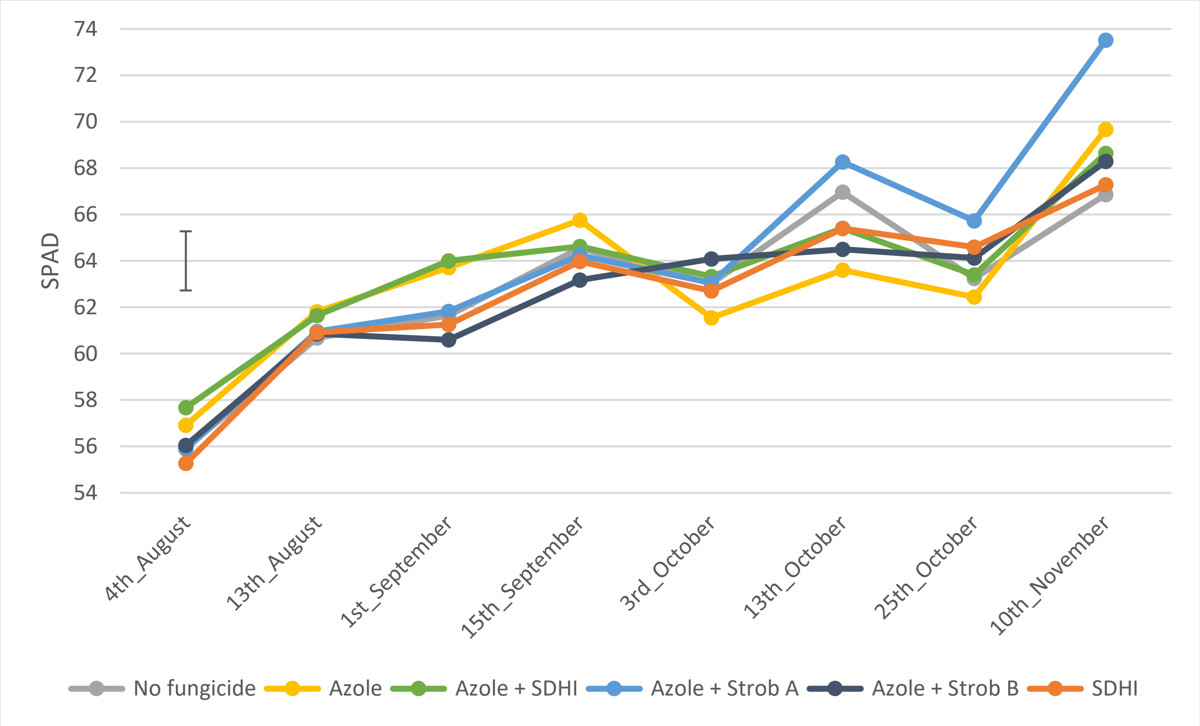


*Figure 3.6 Comparison of SPAD values throughout the 2022 season, including all four varieties used in the experiment, and all dates after the first fungicide treatment. P = 0.054 for differences between fungicide treatments. Error bar is LSD for fungicide * time repeated measures analysis.*

When only Kortessa and Advena were included in the analysis to mirror the 2021 experiment, the azole + strobilurin **A** treatment had higher SPAD values than the SDHI, azole + strobilurin **B**, the azole, and the no fungicide control (**Figure 3.7**, p = 0.056). In the period of the season from August to early September, the highest SPAD values were for plants treated with the azole + SDHI and the azole, but later in the season the azole + strobilurin **A** treatment far exceeded the others.

Table 3.3 Duncan's multiple range test results from a multiple comparisons analysis of SPAD values in the 2022 season, including only Kortessa and Advena. These values are averaged across all dates, and both varieties.

Treatment		**Mean**	**Duncan's multiple range test**
Azole + strobilurin A	🟦	65.28	b
Azole + SDHI	🟩	64.16	ab
Azole	🟨	63.66	a
Azole + strobilurin B	⬛	63.56	a
No fungicide	◼	63.47	a
SDHI	🟧	63.09	a

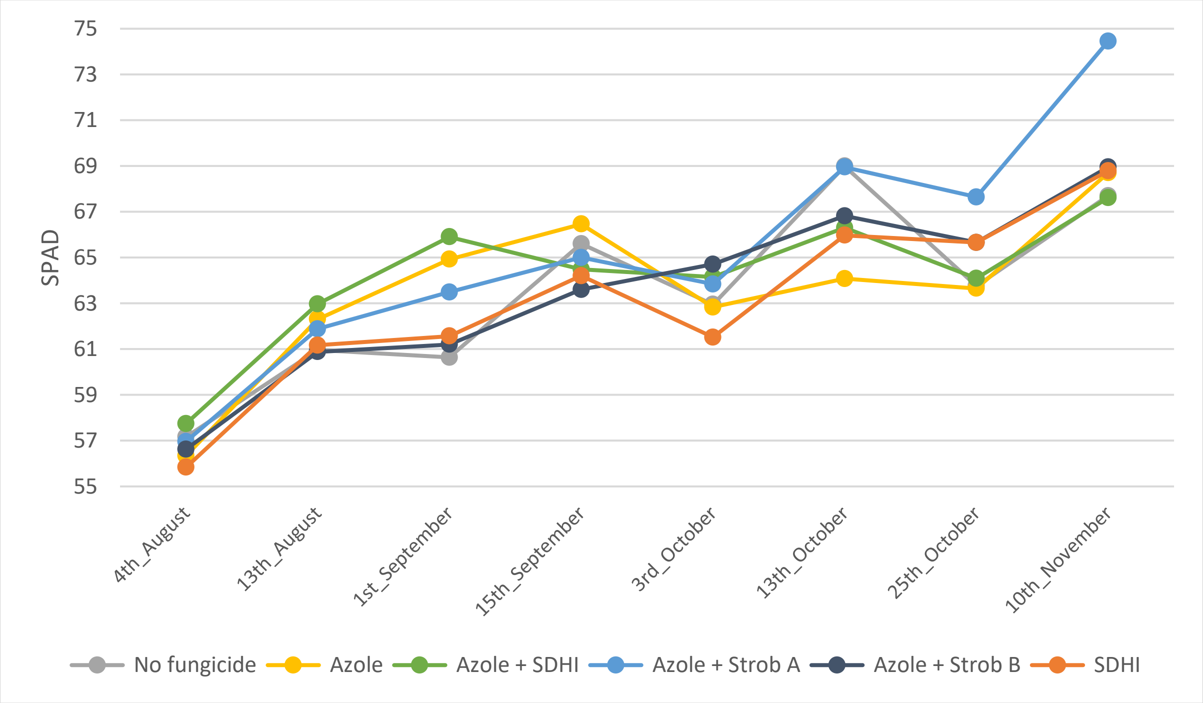


Figure 3.7 Leaf SPAD values from the 2022 season, with only Kortessa and Advena in the analysis. P = 0.056 for differences between fungicide treatments.

Overall, even though in 2022 the statistical significances were reduced compared to the 2021 data, the azole + strobilurin **A** and the azole + SDHI treatments had consistently higher leaf SPAD than the other treatments in both years and regardless of variety.

Compared with 2021, the plants in 2022 had a much larger range of SPAD values, with most values over 60 SPAD units from mid-August onwards, whereas in 2021 the values were never outside of the 50-60 range.

3.3.2 Spectral reflectance indices

3.3.2.1 Triangular vegetation index

In 2021, using a repeated measures analysis, the strobilurin had the highest TrVI value, with the azole + strobilurin combination, and non-treatment control having the lowest values (P=0.009, **Figure 3.8**). This index is positively associated with leaf chlorophyll content. Values were significantly higher for Kortessa than for Advena (p = 0.001), but there was no interaction between fungicide and variety.

Table 3.4 Duncan's multiple range test results from a multiple comparisons analysis of TrVI values in the 2021 season, including both Kortessa and Advena.

Treatment		**Mean TrVI**	**Duncan's multiple range test**
Strobilurin	🟦	2.928	c
Azole + SDHI	🟩	2.881	bc
SDHI	🟧	2.859	bc
Azole	🟨	2.821	abc
No fungicide	▩	2.785	ab
Azole + strobilurin	🟦	2.737	a

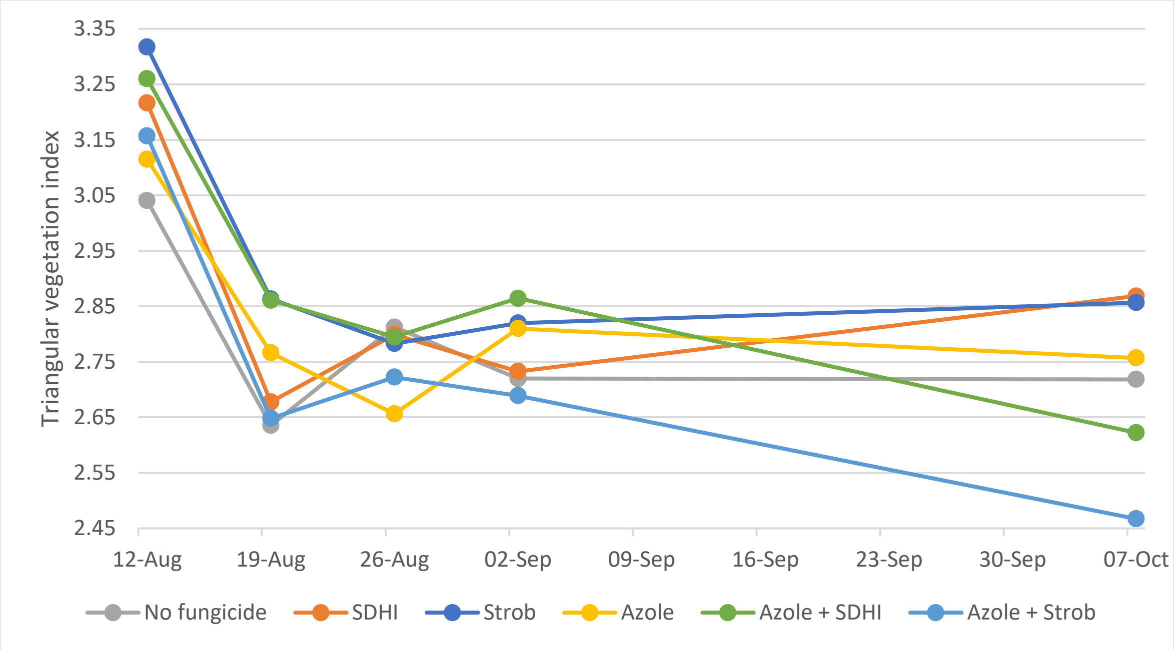


Figure 3.8 Comparison of triangular vegetation index over time depending on the treatment received in the 2021 season. P = 0.009 for differences between fungicide treatments.

3.3.2.2 RARSb

In 2021, the azole + strobilurin combination had higher RARSb values than all other fungicide treatments apart from the no-fungicide control (p=0.021, **Figure 3.9**). This index gives an indication of the chlorophyll b content in leaves, and is positively associated with biomass production. The RARSb values for Advena were higher than those for Kortessa (p=0.003), though there was no interaction between the fungicide and variety on RARSb values, indicating that the effect of fungicides was similar across both varieties.

Table 3.5 Duncan's multiple range test results from a multiple comparisons analysis of RARSb values in the 2021 season. Values represent the mean of Kortessa and Advena.

Treatment		**Mean RARSb**	**Duncan's multiple range test**
Azole + strobilurin	🟦	100.30	b
No fungicide	⬜	96.36	ab
Azole + SDHI	🟩	93.61	a
Azole	🟨	91.69	a
SDHI	🟧	91.62	a
Strobilurin	🟦	91.44	a

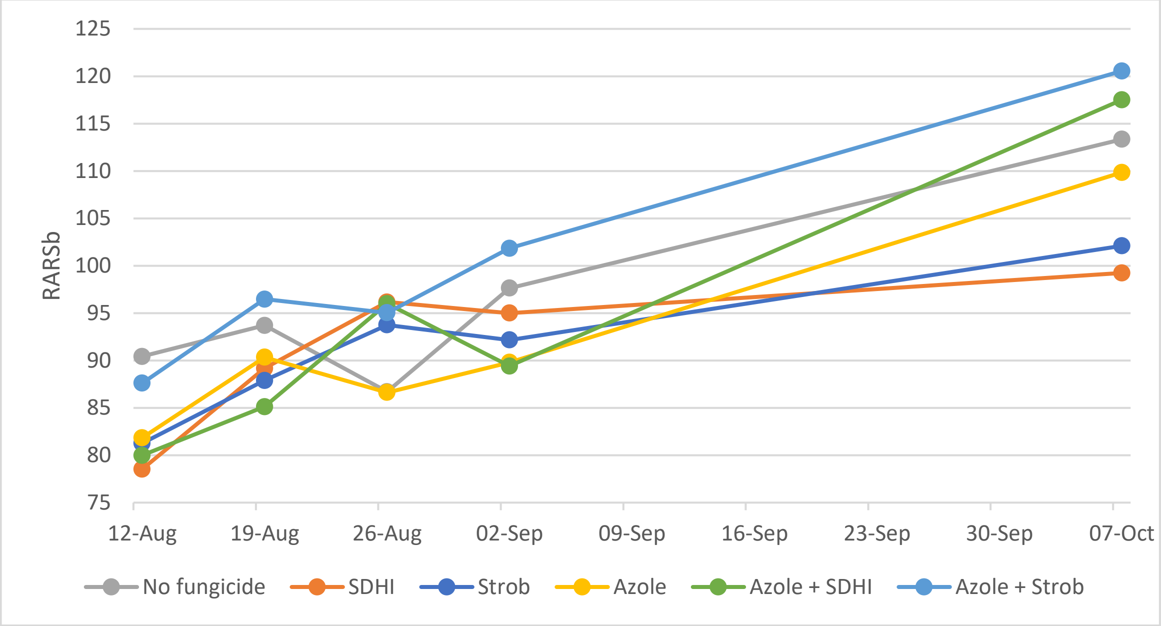


Figure 3.9 RARSb over time for six fungicide treatments in the 2021 season. P = 0.021 for differences between fungicide treatments.

In 2022, there was only one date after fungicide application where data were collected, before the equipment was unavailable for the remainder of the season due to repairs. For many of the reflectance indices, there were differences between the two varieties. However, there were no differences between fungicide treatments, and no interactions found between variety and fungicide treatment for any of the indices.

3.3.3 Canopy cover

When averaged throughout the 2021 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 no fungicide control had a more severe canopy decline than the other treatments (p=0.05 for fungicide effect only, **Figure 3.10**). This was reflected in an interaction between the fungicide treatment and assessment time (p=0.017 for fungicide + time interaction).

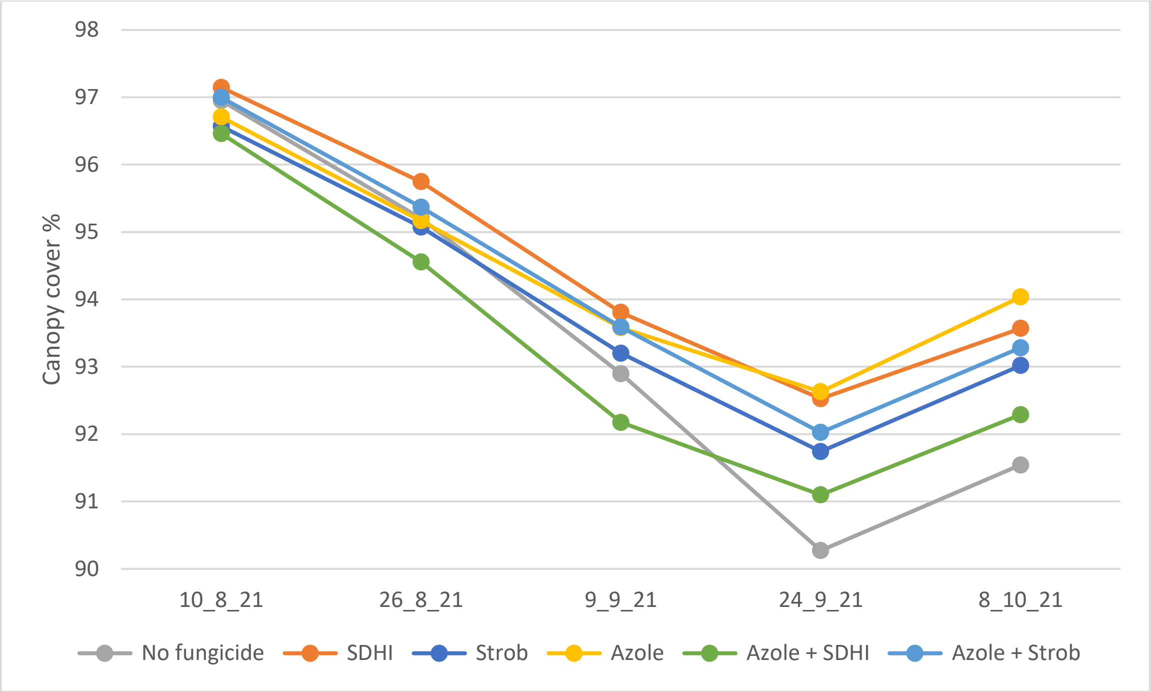


Figure 3.10 Comparison of canopy cover % in plots treated with various fungicide treatments, only including dates after initial treatment, during the 2021 season. P = 0.05 for differences between fungicide treatments.

In the 2022 season, there were no significant differences between the canopy cover % of the different fungicides (p=0.663, **Figure 3.11**). As this season was affected by drought, the plants were undergoing canopy regrowth rather than the expected canopy decline at this stage in the season. To quantify this, in 2021, the total rainfall in July and August was 103.8 mm, whereas in 2022 this value was 29.6 mm (Met Office Sutton Bonington station data).

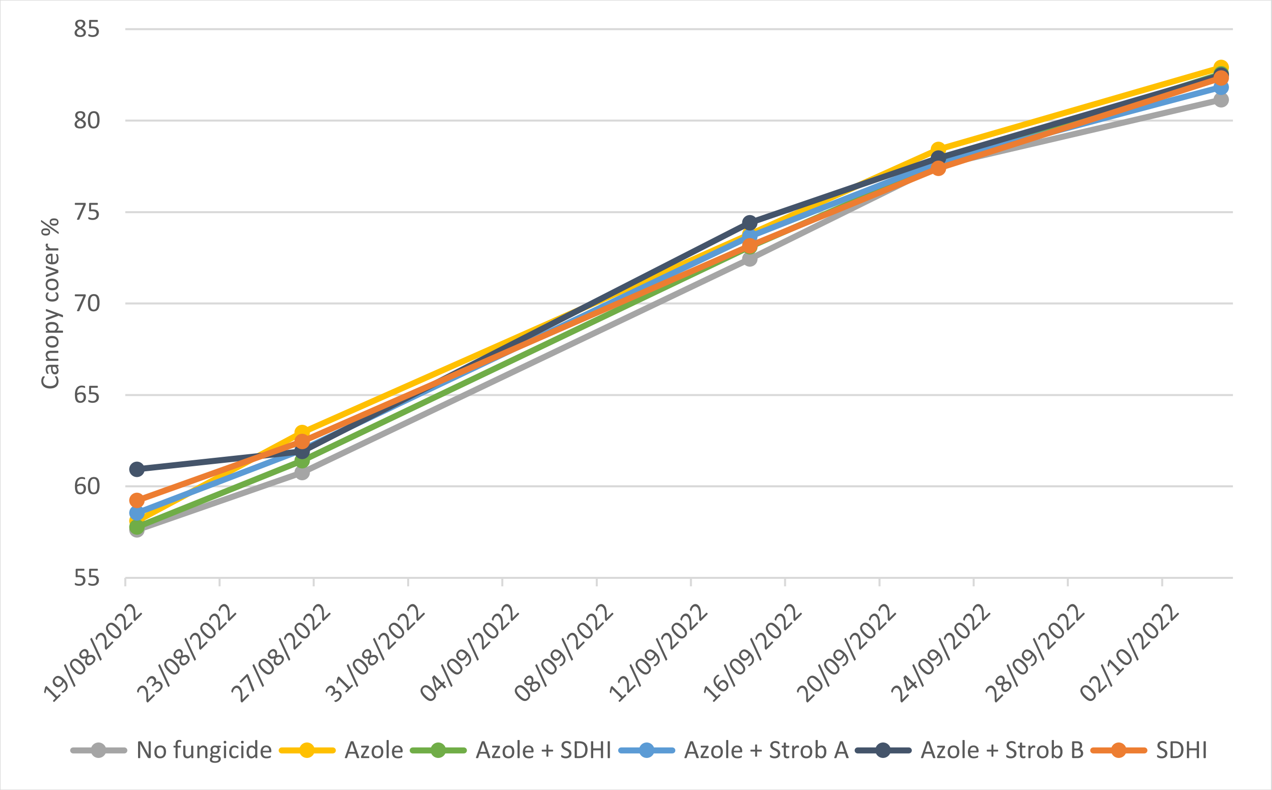


Figure 3.11 Canopy cover % in plots treated with various fungicide treatments, only including dates after initial treatment, during the 2022 season. No significant differences found.

3.3.4 Crop Circle spectral reflectance indices

3.3.4.1 NDRE

In 2021, the lowest NDRE values ranged from the non-treated control at 0.347 to the azole + strobilurin combination at 0.364 (p=0.021, **Figure 3.12**). NDRE gives an indication of the area of healthy vegetation, associating closely with chlorophyll content.

Treatment		**Mean**	**Duncan's multiple range test**
Azole + strobilurin	🟦	0.3635	c
Azole	🟨	0.3612	bc
SDHI	🟧	0.3589	bc
Strobilurin	🟦	0.3541	abc
Azole + SDHI	🟩	0.3503	ab
No fungicide	⬜	0.3474	a

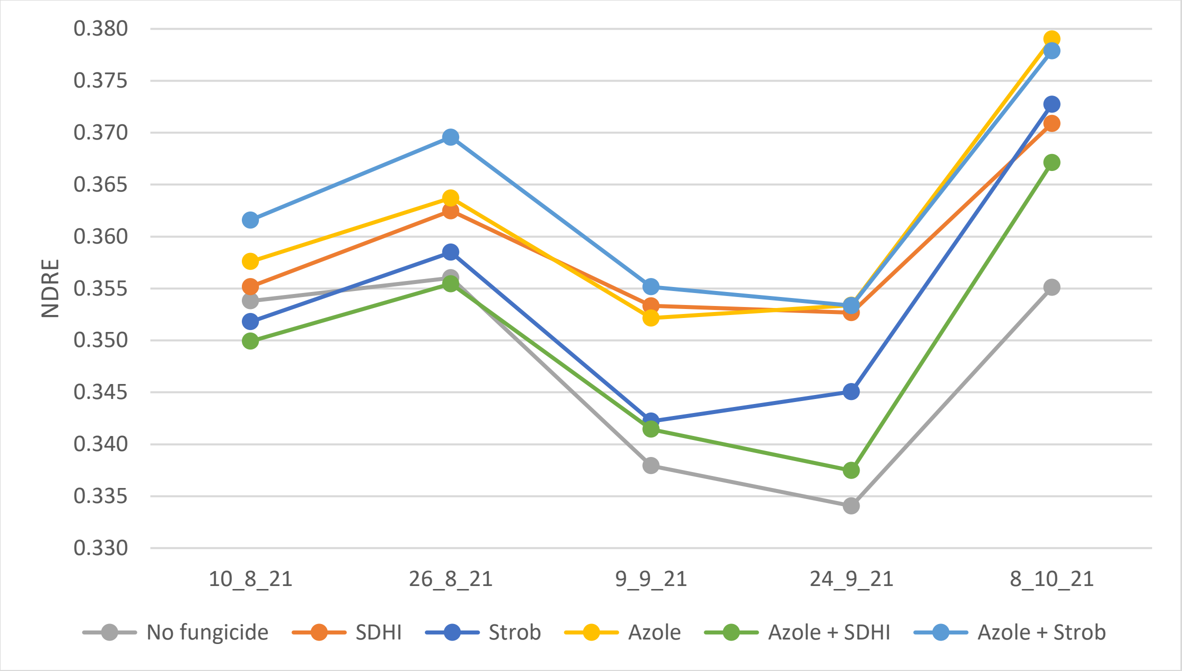


Figure 3.12 NDRE for six fungicide treatments over time in 2021. P = 0.021 for differences between fungicide treatments.

In 2022, when including all four varieties in a repeated measures analysis, BTS1140 had significantly lower NDRE than the other varieties (p=0.001), however, there were no significant differences between fungicide treatments. When only Kortessa and Advena were included to mirror the 2021 experiment, there were no significant differences found between varieties nor between fungicide treatments for NDRE.

3.3.4.2 NDVI

In 2021, the azole, the SDHI and the azole + strobilurin combination had higher NDVI than the no fungicide control and the azole + SDHI treatment (p=0.024, **Figure 3.13**). The strobilurin began to catch up with the other treatments later in the season. NDVI provides an estimate of green area of the canopy.

Treatment		**Mean NDVI**	**Duncan's multiple range test**
Azole + strobilurin	🟦	0.8441	b
SDHI	🟧	0.8418	b
Azole	🟨	0.8415	b
Strobilurin	🟦	0.8334	ab
No fungicide	⬜	0.8248	a
Azole + SDHI	🟩	0.8235	a

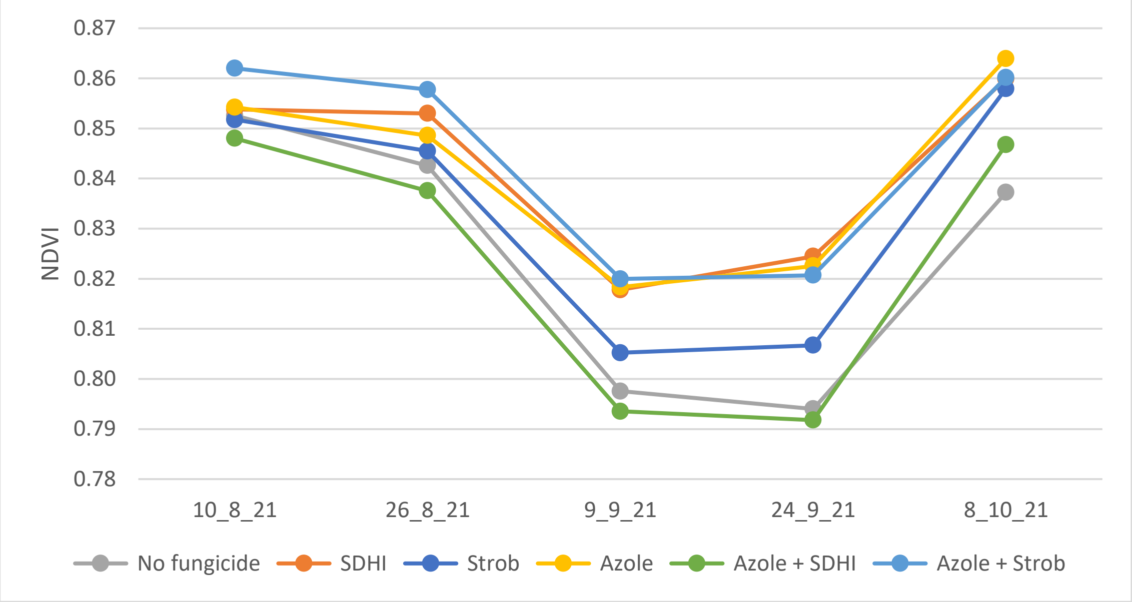


Figure 3.13 NDVI of six fungicide treatments over time in 2021. P = 0.024 for differences between fungicide treatments.

In 2022, there were no statistically significant differences between varieties nor between fungicides for NDVI. This was true in both cases where all varieties were included, as well as when only Kortessa and Advena were included.

3.3.5 Disease assessment

In 2021, when comparing % rust in the plots from the single date measurement, there were 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 no fungicide control had significantly higher rust % than the other fungicide treatments. The interaction is displayed in **Figure 3.14** below. This relationship shows that for Advena, the no fungicide control is highly susceptible to rust, and there was also some disease present in some of the fungicide treatments for this variety, whereas there was minimal rust present across all treatments for the Kortessa variety.

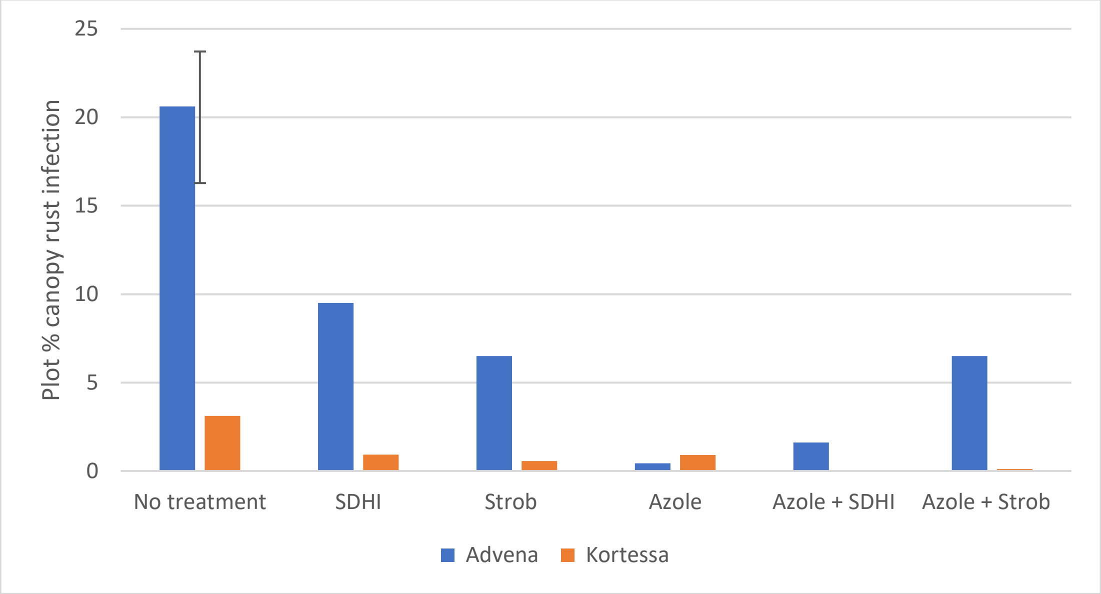


Figure 3.14 Interaction between treatment and variety when comparing rust % in the plots in November 2021. Error bar is the least significant difference. P = 0.026 for the interaction between fungicide and variety.

Powdery mildew was not observed in the 2021 field trial at the time of disease measurements, so no scoring was carried out. Other diseases such as ramularia leaf spot were observed in minor amounts on a very small number of plots.

In 2022, disease scoring was recorded frequently throughout the season. Including all dates and all four varieties in a repeated analysis, the BTS1140 variety had significantly higher rust % scores than the other three varieties (p=0.001). When only including the dates after fungicides were applied, there was a significant difference between fungicide treatments, ranging from between 4% and 18% by the end of the season (**Figure 3.15**, p<0.001), where the untreated control had much higher incidence of rust compared with the other fungicide treatments.

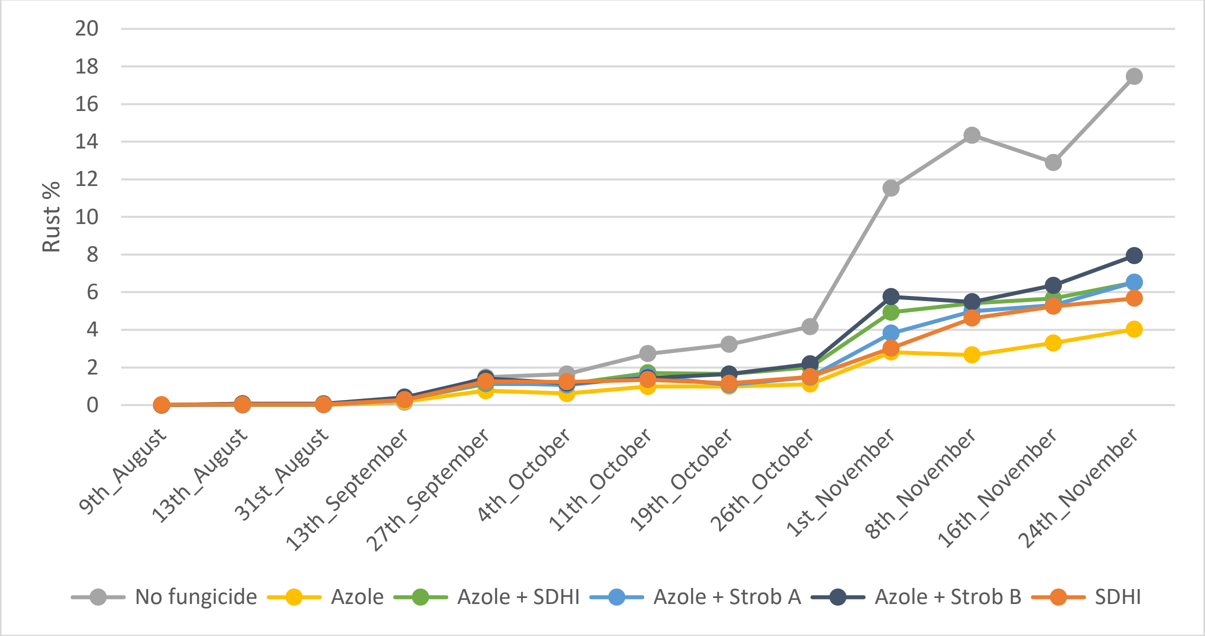


Figure 3.15 Rust % score (% of canopy area showing symptoms) in plots for six fungicide treatments in 2022. P < 0.001 for differences between fungicide treatments.

Cercospora disease was also recorded in the 2022 season, and a repeated measures analysis including all dates and all four varieties revealed that BTS1140 had significantly higher % cercospora score (percentage of canopy area showing symptoms) than the other varieties (p<0.001). When only the dates after first fungicide treatment were included in the analysis, the untreated control had higher cercospora infection than the other fungicide treatments (**Figure 3.16**, p = 0.012).

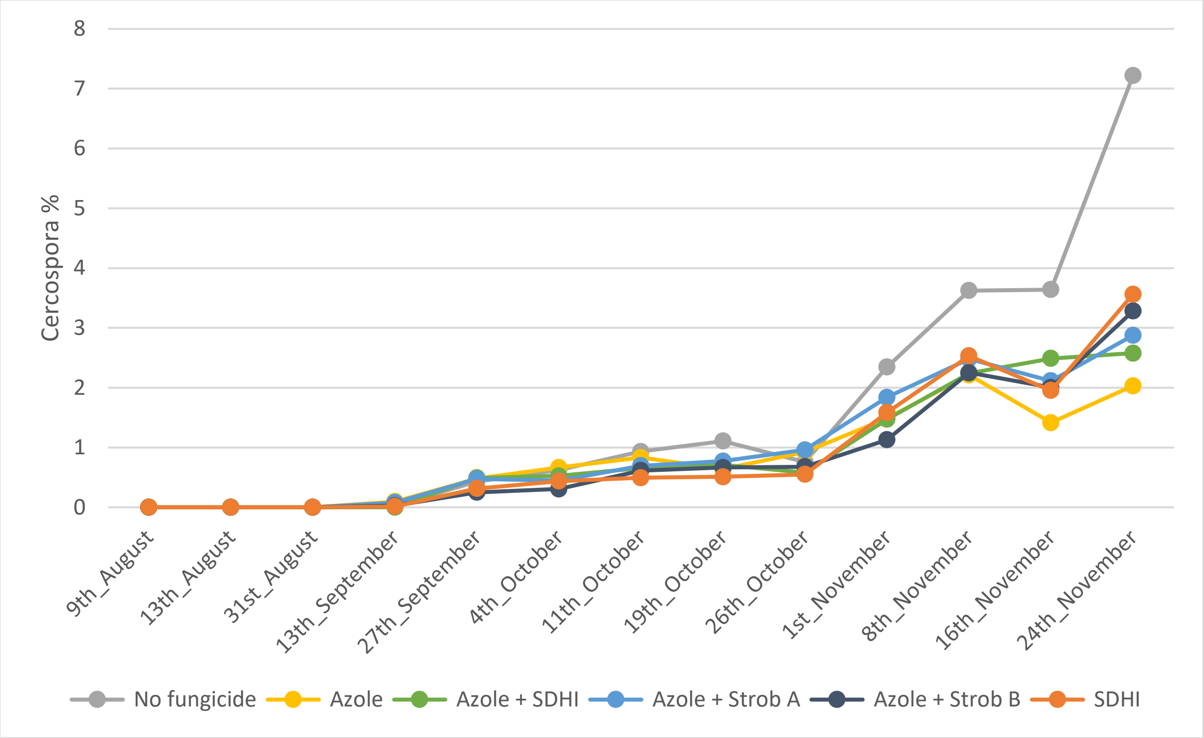


Figure 3.16 Cercospora % score for six fungicide treatments in 2022. P = 0.012 for differences between fungicide treatments.

3.3.6 Harvest data

In the 2021 harvest, there was a trend for differences between fungicide treatments for leaf area (p=0.061), with the non-treated control having smaller leaf area than the treated plants, as shown in **Figure 3.17**.

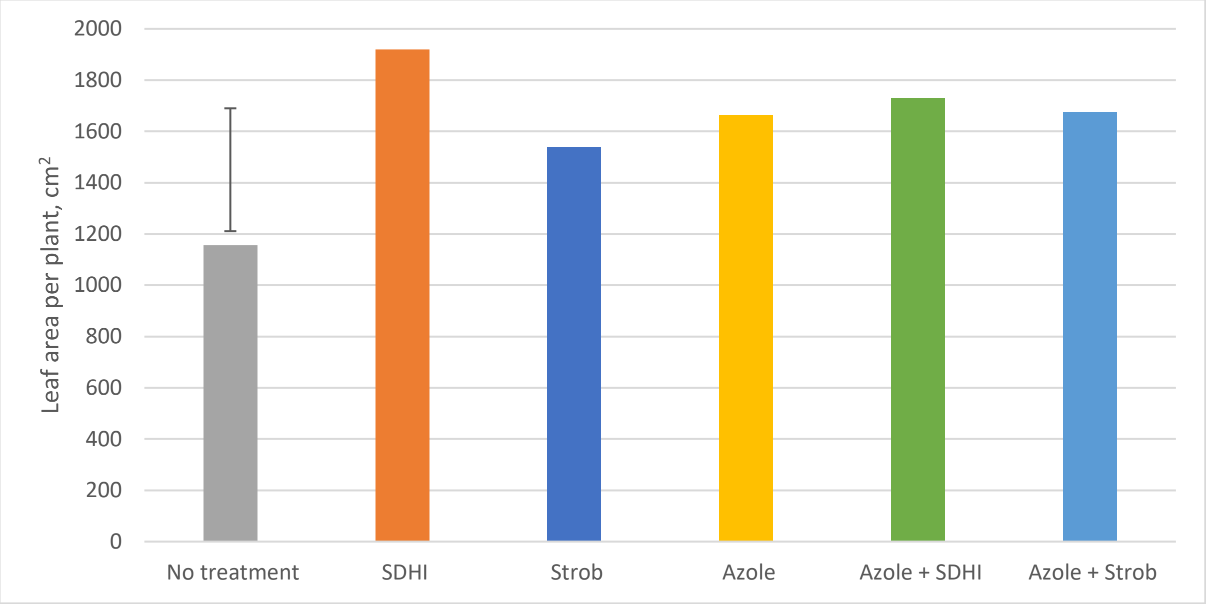


Figure 3.17 Leaf area per plant for six fungicide treatments in 2021. P = 0.061 for differences between fungicide treatments.

In 2021, the azole + strobilurin combination had the higher *specific* leaf weight (weight per unit area of leaf, corresponding to leaf thickness), while the lowest were the SDHI and azole treatments (p=0.05, **Figure 3.18**). These values were calculated using the fresh weight of the canopy at harvest, and the leaf area of those samples. In the 2022 data, this was instead calculated using leaf discs of a specific size and weighing them dried, which is likely to be more accurate.

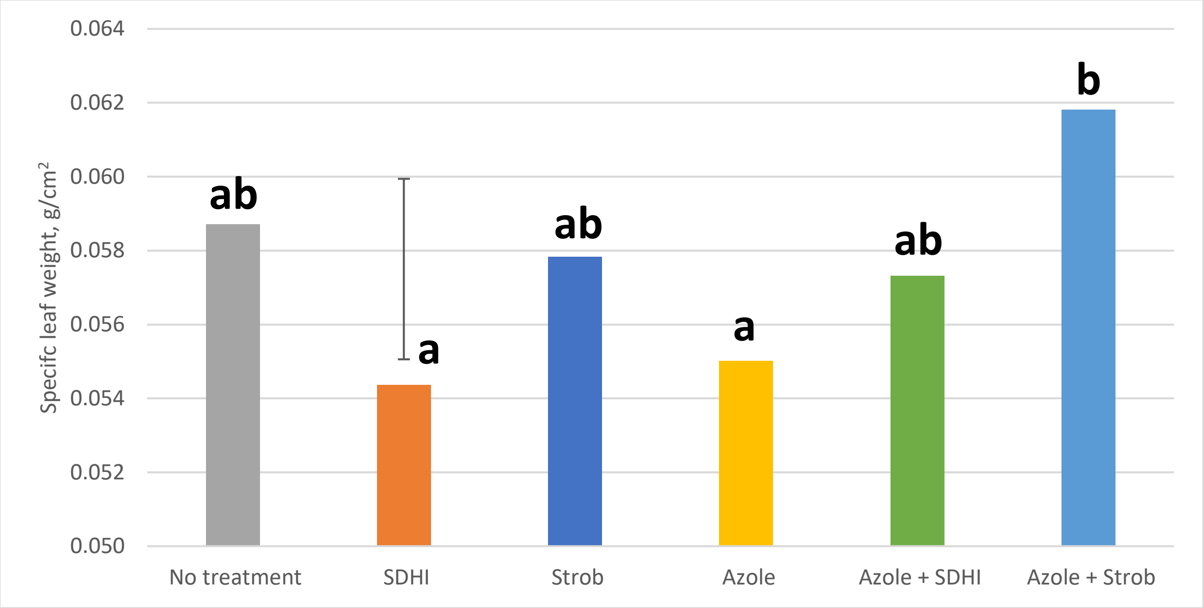


Figure 3.18 Specific leaf fresh weight between six fungicide treatments in 2021. P = 0.05 for differences between fungicide treatments.

While there were no significant differences between treatments for fresh root weights, nor for sugar % in 2021, there was a significant difference between sugar yield; combining these two variables. 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 3.19**). Sugar yield was significantly higher in Advena than Kortessa, yielding 19.98 and 18.49t/ha respectively (p<0.001).

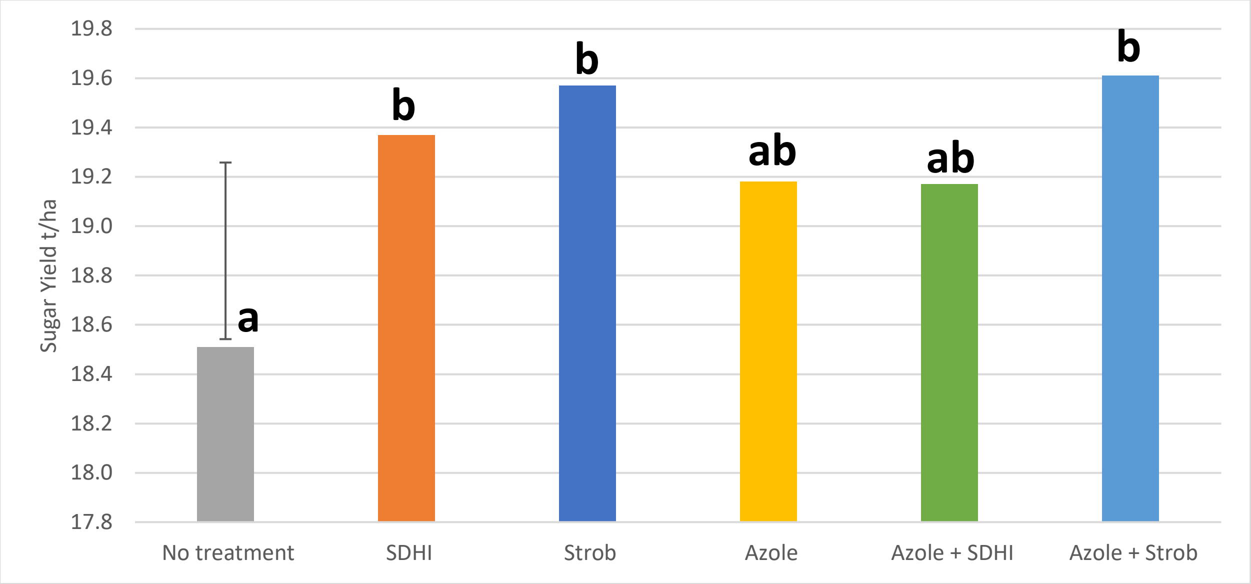


Figure 3.19 Sugar yield for six fungicide treatments in 2021. P = 0.042 for differences between fungicide treatments.

In 2022, when only including Advena and Kortessa in the analysis of sugar yield, there was no significant difference between fungicide treatments (**Figure 3.20**, p=0.124).

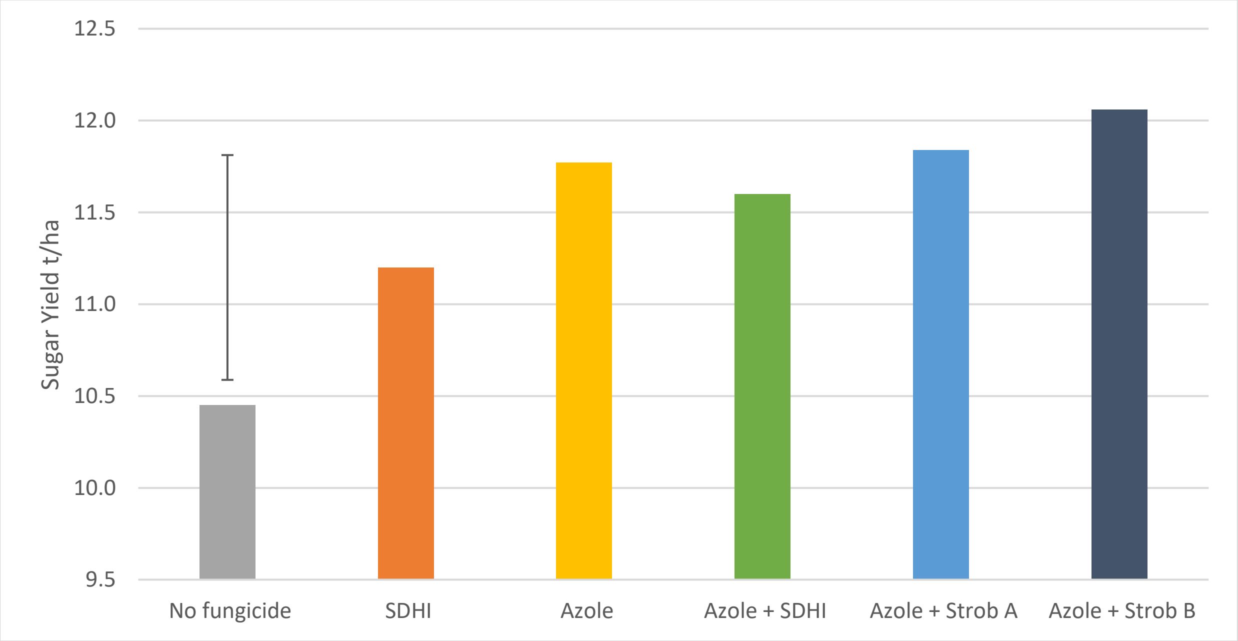


Figure 3.20 Sugar yield of six fungicide treatments in the 2022 harvest, no significant differences (p=0.124).

Although the relationship between fungicide treatment and overall sugar yield was not significant, the relationship with sugar % was much more clear. When only including

Kortessa and Advena in the analysis, the no fungicide control had a significantly lower sugar % than the other treatments (**Figure 3.21**, p=0.006).

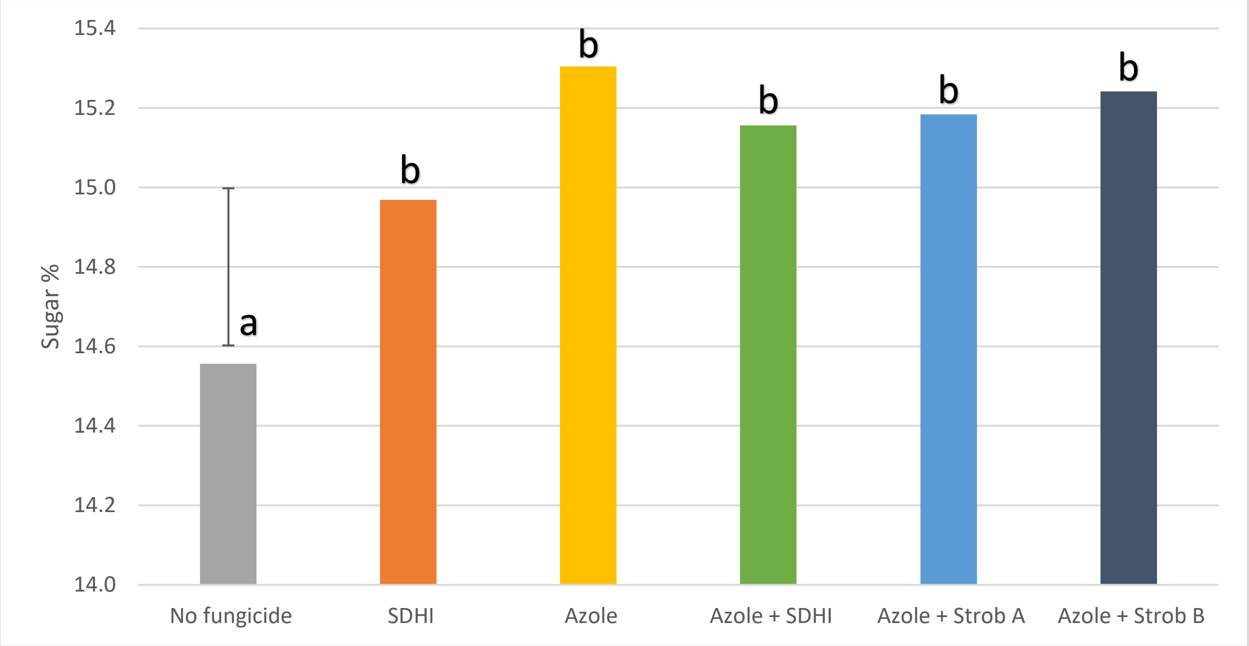


Figure 3.21 Sugar % of six fungicide treatments in the 2022 harvest, p=0.006. Lettering shows significant differences using Duncan's multiple range test.

Before harvest in the 2022 season, leaf discs were collected and their weights recorded both fresh and dried. These values were used to calculate the specific leaf weight in a more accurate way than the 2021 method of recording the whole sample canopy fresh weight and leaf area.

There was a significant difference between fungicide treatments in the specific leaf weight, in which the azole + strobilurin A, the SDHI and the azole had significantly higher specific leaf weight than the no fungicide control (**Figure 3.22**, p=0.003).

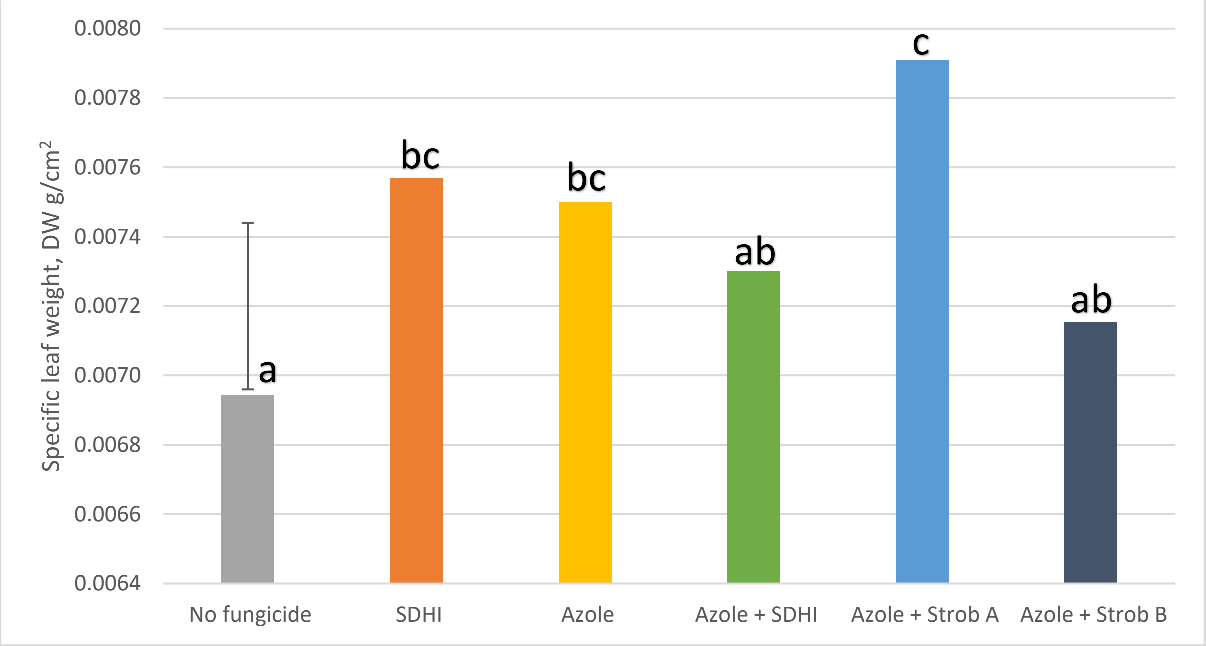


Figure 3.22 Specific leaf weight of six fungicide treatments in 2022 (p=0.003). Lettering shows significant differences using Duncan's multiple range test, and error bar shows the least significant difference.

Additionally to the information about leaf thickness, SPAD data was also collected before leaf discs were taken, so that the relationship between SPAD and leaf thickness could be further clarified. These data suggested that there may be a relationship between leaf thickness and SPAD, which could be as a result of more chlorophyll per unit area in thicker leaves (**Figure 3.23**). As a way to control for leaf thickness, SPAD measurements include the NIR wavelength, which is not absorbed by leaf pigments. For this reason, while SPAD can be used as an approximate estimator of leaf thickness, this cannot be separated from the true amount of chlorophyll per unit area.

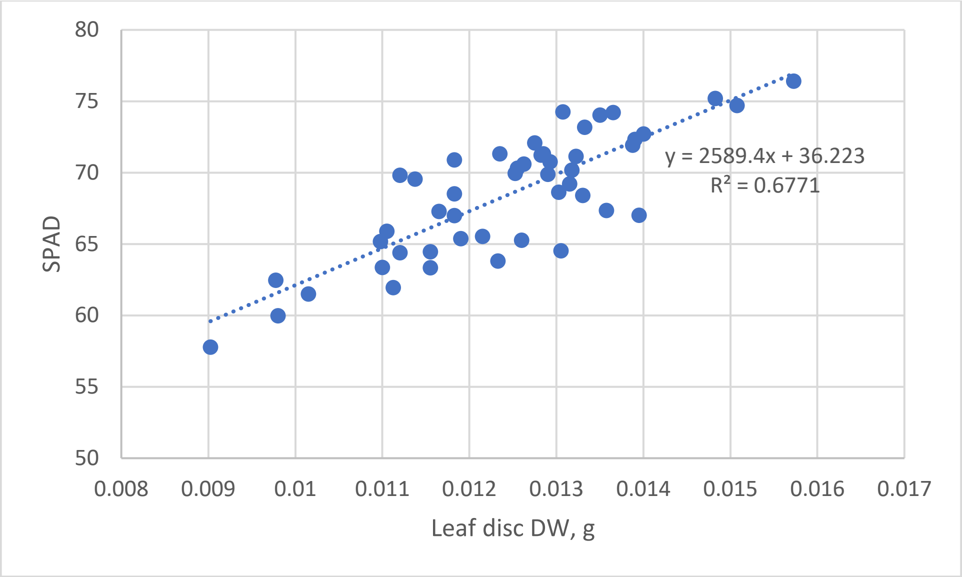


Figure 3.23 Correlation between the SPAD values of leaves and the dry weight of leaf discs taken from the same area of the same leaf.

In 2022, the clean weight of the roots at harvest did not significantly differ between treatments, so the sugar % appeared to have more influence on the total sugar yield in this case. This suggests that the fungicides had little effect on the root biomass growth, but did have an effect on sugar concentration. Similarly, in 2021 there was no significant difference between fungicide treatments for clean root weight, however, there was a trend for differences between treatments for sugar %, although these were not statistically significant (p=0.130).

In 2021 the effect of fungicide treatment on sugar yield was significant (p=0.042), but not in 2022 (p=0.124). In both years, the lowest yielding treatment was the no fungicide control, and the highest yielding treatments were the azole + strobilurin treatments.

3.4. Discussion

The field experiment was much larger than the polytunnel experiment in the same year (drought experiment, **chapter 2**) and this allowed for greater consistency across more treatments, which was reflected in the results. The azole + strobilurin treatment, which is frequently used as standard in sugar beet production, 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 weight (**Figure 3.18**), indicating 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 weight, 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 in **Chapter 2**, 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, these 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 – not to be totally discarded.

This experiment allowed the comparison between varieties of different disease susceptibility, and while there were some differences between varieties for some physiological traits, which may reflect the effects of pathogen control, there was no interaction for the traits which improved confidence that the effects of the fungicides observed are similar across the varieties. If the positive responses had only been observed in the disease susceptible variety, this would have indicated that the effects were primarily due to pre-symptomatic disease control rather than extra physiological benefits.

3.4.1 Comparing leaf SPAD between treatments

Some measurements showed similar effects across both years of the experiment, while others showed differences between the two years. When looking at leaf SPAD, which provides an indication of leaf greenness and chlorophyll content, two fungicide treatments were associated with highest SPAD values across both years; the azole + strobilurin **A**, and the azole + SDHI. Interestingly, the azole only treatment had the lowest SPAD of all treatments in 2021, but it was not significantly different from the top treatments in the 2022 experiment. This showed that responses varied highly between the years for some treatments. The reasons for this can not be certain, but could be associated with yearly differences in disease pressure and weather.

SPAD can be higher in drought-stressed plants, where leaf expansion is restricted and the components within the leaf become more compressed, so the drought which occurred in 2022 could have affected results, especially if any of the fungicides affected the plant’s responses to drought. It would be valuable to repeat this experiment in conditions without drought stress, to determine if this had been a factor in some of the differences between the two years of this experiment.

It is important to note that even small SPAD values indicate leaves which are quite green, and therefore any numerical differences, including those which may be statistically significant, may have a limited impact on final yield, as chlorophyll content was unlikely to be a limiting factor in any treatments.

3.4.2 Comparing other reflectance indices between treatments

In 2021, there were some significant differences between fungicide treatments when looking at canopy reflectance indices, including TrVI, RARSb, NDVI and NDRE. However, no differences were found between treatments in 2022. The drought conditions in 2022 could have contributed to this, as the canopy rapidly declined after the onset of drought, affecting the subsequent growth of the plants.

In 2021, the azole + strobilurin **A** combination was the highest performing treatment for RARSb, NDRE, and NDVI, suggesting that the canopy had a larger active green area than other treatments. This azole + strobilurin **A** combination was also the highest sugar yielding treatment that year, which improves confidence that these reflectance indices can indicate improved photosynthetic capacity.

Although there were not statistically significant differences in sugar yields between fungicide treatments in 2022, the azole + strobilurin treatments (both **A** and **B**) were the highest yielding treatments, which indicates that there was some consistency between the two years. The azole + strobilurin **A** treatment also had the highest specific leaf weight across both years of the experiment, indicating that the leaves were thicker than for other treatments. Thicker leaves could be related to more chlorophyll and Rubisco per unit area and a higher leaf photosynthetic rate, which could be one of the contributors to the increased yield.

In 2021, the no fungicide control was amongst the lowest SPAD values, TrVI, canopy %, NDRE and NDVI, and also resulted in the lowest sugar yield.

3.4.3 Canopy persistence and stay-green effect

The green canopy area and its persistence were measured through various spectral reflectance methods. These data can contribute to understanding how the stay-green effect and delayed senescence affected biomass growth. The rate of canopy decline and senescence is relevant due to the abundance of research demonstrating a relationship between strobilurins and delayed senescence and the stay-green effect.

It's important to consider the concept that a stay-green effect may be visual, but for it to contribute to improved yields, the effect must be functional. For the effect to be functional, the canopy must not only *appear* greener than the untreated plants, but also remain photosynthetically active for a longer period. Additionally, it must be considered that any delayed senescence, including functional stay green, would only result in improved yields during the period of sugar yield formation before storage ends or significantly decreases during this time. This window of yield formation and storage differs between various crops, and so the relevance of this would need to be fitted to the current understanding of sugar beet yield formation.

Sugar beet plants continue to produce and store sugar well into the season, with sugar still being produced and stored in the later months of growth. This makes it even more relevant to identify ways to extend the period when photosynthetic capacity is optimal, including fungicides which produce a stay-green effect, as this could be a major factor in improving yields.

During this experiment, canopy cover % provided an insight into canopy expansion and decline, but as this factor is limited to quantifying all green area, it doesn't provide certainty that any extended greenness is functional. For this reason, it is important to supplement the canopy cover % data with multispectral reflectance data, including NDVI and NDRE, as these are more likely to quantify chlorophyll content relating to functional green area than canopy area alone.

3.4.3.1 The specifics of delaying senescence

Several studies have explored the physiological processes underlying delayed senescence with fungicide use. Grossmann et al. (1999) explored the effects of the strobilurin fungicide kresoxim-methyl on wheat leaf senescence, and reported the regulation of phytohormone levels during the experiment. Plants treated with this strobilurin fungicide had delayed senescence, a reduction in ethylene production, and an increase in cytokinins in leaves, which tend to be associated with a prolonged leaf photosynthetic period. The responses of phytohormones to drought stress response and fungicide use are explored in the context of drought in **Chapter 2** of this thesis. However, this section will focus on how they respond to fungicide use during standard senescence, in absence of additional stress.

The relationship between strobilurins and ethylene production has also been demonstrated in tomato plants, where strobilurin application was shown to decrease ethylene content in the flowers, which resulted in improved yields by reducing flower drop (Giuliani et al., 2019).

A study explored the effect of bixafen, a pyrazole carboxamide fungicide, on the senescence and yields of wheat in absence of disease, and found that leaf senescence was delayed in treated plants, and yields were subsequently improved (Berdugo et al., 2012). In this study, leaf temperature was recorded as an indicator of leaf transpiration and photosynthetic activity, and treated plants had lower temperatures than untreated plants. This provides some more context to the concept of functional stay-green effect, where the effect was not just visual but the health and photosynthetic activity of the leaves was truly improved.

While reduced ethylene production has been demonstrated to be one of the major contributing factors to delayed senescence under fungicide treatment, other factors have been explored which could have a major role in the interference of the senescence process. A study explored the antioxidant activity in spring wheat during senescence, and demonstrated that fungicide-treated plants resulted in increased antioxidant activity, slowing down the process of senescence (Wu & von Tiedemann, 2001). Specifically, the level of superoxide ($O_2^{\bullet -}$) which is normally increased during senescence was reduced by fungicide application, and the activity of the antioxidant enzyme superoxide dismutase (SOD) which is normally decreased during senescence was increased. These observations suggest that fungicide-induced delay of senescence is at least in part due to enhanced antioxidative activity.

3.4.4 Disease as a contributing factor

The disease scoring revealed that the no fungicide control had higher disease incidence than the other treatments, which could have been a major contributing factor to the impacted canopy reflectance indices. By including two varieties of differing disease susceptibility, there is improved confidence that the physiological benefits of the fungicides are a significant contributing factor with reference to the responses of the resistant variety.

Disease control is a major factor which must be explored in this experiment, in order to determine the extent to which improved physiology and yields may be due to disease control rather than extra physiological responses. In 2022, the untreated control consistently had higher rust and cercospora incidence than any other fungicide treatments, and this was also seen on the single date that disease was recorded late in the 2021 season. While Kortessa did have lower disease incidence than Advena, they both displayed the expected relationship where the untreated control displayed more disease than the other fungicide treatments. This provides some confidence that any physiological benefits which were observed under either variety were at least in part due to physiological benefits of fungicides and not due to disease control alone. To determine the extent to which early disease control contributes to improved green area, photosynthetic capacity and yield, a further experiment in controlled environment conditions in the absence of disease would be valuable. The scale of this experiment meant that it was not feasible to replicate in controlled conditions in this project, but a smaller-scale controlled experiment at the canopy scale would still give some confirmation to the present results. Additionally, if this topic is explored further, using a larger range of varieties with differing disease susceptibilities would provide more information on the extent to which disease control may have contributed to the improved expression of physiological traits and yields.

3.4.5 Economic benefit of fungicides in absence of disease

While interpreting the results of this study, an important concept to consider is the economic benefit of using fungicides for their physiological benefits, in the absence of disease, or in early treatments where disease has been predicted but not yet detected. Current legislation in the UK permits fungicides to be applied to crops at the first sighting of disease, not before. This legislation points to an important consideration, that any additional chemical application to crops comes with a potential environmental cost. While any negative impact is difficult to quantify, all products undergo tests during the process of obtaining registration, and overall environmental impact of product usage should be included in cost/benefit analysis. If permissions change and these chemicals can be used preventatively or for their physiological benefits alone, more focus will turn to the economic benefit of using such chemicals in the absence of disease. When exploring this concept, two major factors have to be considered; yield penalty through early application of fungicides, and profitability of fungicide application in the absence of disease. The following sections explore these two concepts.

3.4.5.1 Deleterious effects of fungicide application

A major topic to consider when drawing together conclusions about the physiological benefits of using fungicides is that there is some evidence that in certain contexts, fungicide application can actually lead to a reduction in yields.

A study on the effects of fungicides on soybean crops found that treatments containing tebuconazole resulted in mild phytotoxicity in plants, although it was noted that this could be due to a number of reasons, including elevated temperatures during application, plants under water stress, or elevated dosage of the products (Zuntini et al., 2019). Additionally, it was proposed that tebuconazole in particular may have interfered more with the plant physiology in this experiment than other members of the triazole group because it has comparatively faster absorption and slower translocation through vascular bundles, making it more likely to accumulate in leaves than other treatments. This increased interference was also suggested to be more likely under conditions where product is applied during high temperatures, and that elevated dose of the product can also increase risk of phytotoxicity.

Many triazole products have been shown to inhibit plant growth in certain conditions, due to their inhibitory effect on gibberellin biosynthesis, however, these growth regulatory effects can result in beneficial outcomes such as reduced lodging (Berry & Spink, 2009). An important concept to consider in contrast to this section is that the interaction between fungicides and crops may have temporary negative effects, or seemingly harmful effects, which actually benefit the crop as a whole. Reduced growth resulting in a lower risk of lodging is just one example of this, but this concept also applies when considering **Chapter 2**, where a product impacting a plants stress response might be seen as damaging, but improves the ability of the crop to continue activity through temporary periods of stress.

3.4.5.2 Fungicide costs and profitability

This concept has been studied in winter wheat (*Triticum aestivum*), with conclusions that there was no economic benefit to fungicide application in the absence of disease, as fungicide costs would have to be lower than the cheapest fungicide in the study for more than a 50% chance to break even or result in a profit (Weisz et al., 2011). This cost analysis incorporated a grain price range, fungicide application costs, and loss of yield through driving over wheat during application, to find a value for the yield increase required to pay for a fungicide application. This was modelled by calculating this value for each combination of costs and grain prices to find the probability of breaking even in each.

In many crops, sugar beet included, untreated disease can lead to such large yield losses that fungicide usage is inevitably more profitable than not treating disease. While it's important to consider the profitability of fungicide use in absence of disease for the purpose of this research, in practice the crop will not realistically be without any risk of disease for the entire season. For this reason, most sugar beet growers would always apply a fungicide onto the crop at some stage during the growing season. The focus therefore turns to the period of the season in which disease has not yet appeared, and whether it would be profitable to treat plants during this time, if such usage becomes permitted.

3.4.6 Experimental limitations

A limitation of this study was that disease scoring was only carried out on one date in 2021, so it's difficult to compare accurately between the two years of the experiment. In 2022, disease scoring was regular and allowed for the changes over time to be more clear, so this would've been a helpful source of data for comparison.

A further limitation was that the hyperspectral radiometer was under repair for much of 2022, which meant that reflectance data were limited to NDRE and NDVI. It would have been useful to gain a larger selection of reflectance indices, and to compare between the two years.

An additional limitation of this study was that it could have been valuable to include two forms of control, rather than just one. For this experiment, the control put in place to determine physiological benefits additional to disease control was to use two varieties with differing disease susceptibility. It could have improved understanding to have also included a fungicide which is known to control disease without having any additional physiological effects, such as chlorothalonil.

The final limitation to consider is that much of the UK suffered a period of drought and high temperatures in the summer of the 2022 season. This makes it more difficult to separate out the general effects of fungicides on sugar beet physiology, from the effects of such products on abiotic stress responses. The purpose of this trial was to observe the changes which occur in sugar beet in standard field conditions under a range of fungicide treatments, without interference from stress factors. However, even with the impact of drought stress, some relationships were similar across the two years and differing conditions are a reality of field experiments.

3.4.7 Conclusions

This experiment compared a range of fungicides applied to varieties of sugar beet with differing disease susceptibility. While some relationships were not seen in both years, some relationships were consistent. In both years, an azole + strobilurin treatment was amongst the highest performing and amongst the highest yielding, while the untreated control was consistently lower than other treatments for physiological trait indicators and yields. While specific leaf weight was not directly correlated with increased yields, the highest leaf weight was consistently found to be of the azole + strobilurin treated plants, so this may be an important factor in improving photosynthetic capacity. While the different varieties sometimes had different physiological trait measurements, there were no interactions between fungicide and variety, so the effects of fungicides were similar across the varieties. This improves confidence that the effects seen by fungicide use are not exclusively due to disease control.

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Chapter 4. The effect of fungicides on sugar beet's response to nitrogen limitation.

Abstract

Sugar beet growth is heavily dependent on nitrogen availability in the soil, with optimum fertiliser amounts of approximately 120kg N/ha of nitrogen in the UK. Below this amount, leaf expansion and canopy closure is slowed, and subsequent sugar yields are decreased. Above this amount, yields can also be impacted by changes in partitioning where canopy growth is prioritised over root expansion and sugar storage, as well as increased impurity content in the root which decreases extractability of sugar and subsequent yields. In the UK, it is standard practice to apply nitrogen fertiliser to the soil early in the growing season to provide the optimum nitrogen availability. Published studies in other crops have indicated that fungicide applications can improve nitrogen uptake and metabolism, allowing plants to cope in low nitrogen soil if they have had certain fungicides applied. This relationship has not yet been published in sugar beet. In this experiment, sugar beet physiology and yields were compared between conditions of sufficient nitrogen fertiliser and limited nitrogen. This study found that nitrogen limitation had significant impacts on physiological traits and yield performance of sugar beet. The interaction between fungicide application and nitrogen level on these traits was inconsistent across experiments.

4.1. Introduction

4.1.1 Nitrogen in plants

Nitrogen is essential for growth and development in crops, and its availability in soil affects plant processes including leaf expansion, photosynthesis and yields. Several plant molecules essential to photosynthesis contain nitrogen in their structure, including essential amino acids, phytohormones, ATP (adenosine triphosphate) and chlorophyll (Crawford & Forde, 2002).

Additionally, nitrogen has also been shown to improve the resistance of some crops to environmental stresses, including high soil salinity (Chen et al., 2010) and early stages of drought stress (Olšovská et al., 2024). Interestingly, Olšovská et al. (2024) found that while high levels of nitrogen fertiliser were beneficial for drought stress tolerance in barley during early growth stages, it had detrimental effects during subsequent growth stages. These conclusions emphasise the importance of finding a suitable balance and optimum nitrogen dosage, considering abiotic stress factors.

Plants uptake nitrogen in the form of nitrate and ammonium ions, which are absorbed alongside water via the plants roots. Nitrate is then transported to the cytosolic compartments of plant cells, where it is reduced to nitrite by the enzyme nitrate reductase. Transporters then deliver nitrite to the chloroplast, where the enzyme nitrite reductase reduces it to ammonium. This process is outlined in the figure below (**Figure 4.1**).

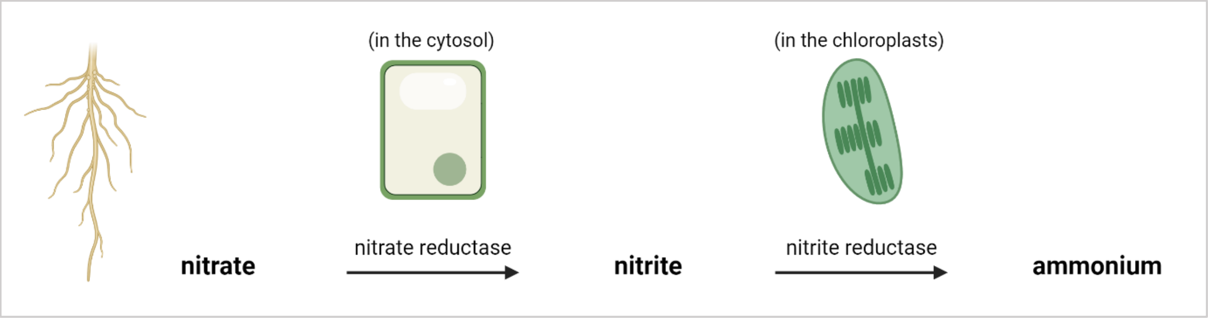

Figure 4.1 Overview of the process of nitrate uptake, transport and reduction in plants.

Nitrogen fertiliser is routinely applied to the soil for crops such as sugar beet, in order to increase the amount of nitrogen available to the crop. However, this can become an expensive intervention if soils have low nitrogen and require a large amount. Compost and animal manure have historically been used to improve the fertility of soils, and are still frequently used as common practice although they release nitrogen more slowly than inorganic fertilisers. Additionally to these organic fertiliser methods, synthetically produced fertilisers have become increasingly common since their development. Many farmers use a combination of manures and synthetically produced fertilisers for their soils.

As well as applying nitrogen fertiliser, there are some additional ways that farmers can improve nitrogen availability in soils. One method which can be used is growing legume crops within the rotation, especially before crops which may require more nitrogen to perform well. This method can also be applied in the form of a cover crop, rather than limiting the use of legume plants to previous crop rotations. Some examples of plants which are routinely used as cover crops include fodder vetch (*Vicia villosa*) and various clovers such as red clover (*Trifolium pratense*) and white clover (*Trifolium repens*). These legume plants can successfully fix nitrogen from the air into the soil, using Rhizobium bacteria which are found in the root system of the plants.

Improving soil structure and health can also have significant impacts on nitrogen availability. For example, soils with compaction issues can lead to poor root growth and reduced water infiltration and drainage, which can all result in plants being unable to access and take up nitrogen efficiently. There are many factors which can improve soil structure, such as regularly adding organic matter including composted manure, and avoiding overworking the soil or compacting the soil in poor conditions.

Soil pH can also affect nutrient uptake, with particularly acidic soils leading to nutrients being wasted as they are not able to be taken up by the crop. Typically this issue is resolved by applying a specified amount of lime to correct the pH, depending on how low the recorded pH is (Holland et al., 2018).

4.1.2 Nitrogen usage in sugar beet

Uptake of nitrogen is vital 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 approximately 3, which will intercept 95% of incoming radiation (Hoffmann, 2019). To achieve this, the crop tends to require approximately 90-120kg N/ha of fertiliser. If the nitrogen dosage increases above this amount, the N response plateaus, where any further increases do not result in yield improvements.

Above the optimum N rate, if plants are supplied with an excessive application of fertiliser, 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, resulting in reduced sugar yields. In some countries, the optimal nitrogen dose is different to the UK, depending on a range of factors including soil types, weather conditions, temperatures, and growth seasons.

This concept of optimal fertilisation is demonstrated in **Figure 4.2** below, in a resource generated by a meta-analysis of sugar beet yield responses to nitrogen fertiliser (Jaggard et al., 2009).

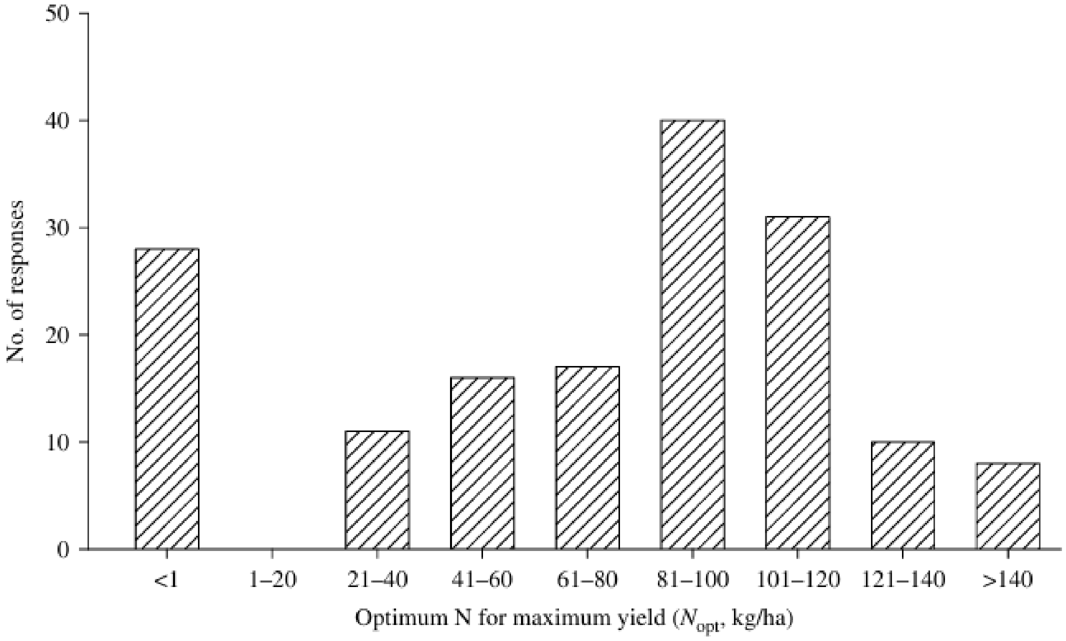

Figure 4.2 Graph demonstrating the frequency distribution of the optimum nitrogen fertiliser for maximum sugar beet yield, generated by a meta-analysis of sugar beet yield responses to nitrogen fertiliser (Jaggard et al., 2009).

There are many factors that can contribute to lower nitrogen availability in soils. One leading factor is the soil type, where sandy and well-drained soils typically leach nutrients faster than other soil types. Heavier clay soils can also be at risk of lower nitrogen availability, as they can be more prone to becoming waterlogged, a risk factor for denitrification, where nitrate and nitrite are converted to gaseous forms of nitrogen by anaerobic soil bacteria and returned to the atmosphere. Soil moisture can also impact nitrogen availability, as excessive irrigation or heavy rainfall can cause nitrogen to leach out of the soil with the water, and insufficient watering can be detrimental to the absorption of water-soluble nutrients. In the UK, most sugar beet is grown on the east side of England, where much of the soil is sandy loam, and therefore at risk of nutrient leaching. This area is also frequently the driest in the UK, receiving less rainfall on average than other regions in a typical year.

4.1.3 Physiological effects of nitrogen limitation

Crop emergence can be significantly impacted by insufficient nitrogen availability in soils, as nitrogen is essential for the production of many vital plant compounds. The effect of insufficient nitrogen can result in slower emergence and growth rates, which has a knock-on effect on plant stand. Where plant stand is impacted, crop cover can become patchy, which impacts total light interception and subsequent yield accumulation. Ultimately, poor crop emergence affects the crop at the beginning of the season, but has knock-on effects throughout the season which result in lower yields.

Insufficient nitrogen availability has been shown to affect many processes which are involved in cell division and leaf expansion. These processes impacted by insufficient nitrogen include DNA synthesis, cell division, and cell growth (Rivin & Fangman, 1980). Further research has shown that nitrate content also affects cytokinin synthesis and distribution, as well as downstream transcription factors, which also impacts the regulation of cell division (Miyawaki et al., 2004). With these impacts on canopy expansion, the resulting effects on the crop are that light interception is reduced, which reduces yield potential. This is especially important in sugar beet, where sowing cannot begin until the weather is mild enough that bolting risk is low, and yields are directionally proportional to light intercepted throughout the season. This presses importance on the canopy to expand quickly and maintain its cover for as long as possible. Nitrogen insufficiency has also been linked to thinner leaves, which can be related to decreased photosynthesis (Isla et al., 2016).

As well as the initial impact of nitrogen insufficiency on canopy area which reduces overall photosynthesis, there is also a direct impact on the photosynthetic processes as a result of insufficient nitrogen.

One process which is involved in photosynthesis and is also affected by nitrogen availability is stomatal regulation. Stomatal regulation is a useful indicator of physiological activity, as this process tightly controls gas exchange in the leaf, and can respond to a range of physiological inputs and stress conditions. Nitrate presence has been shown to drive stomatal closure by promoting the opening of SLAC1, an anion channel which controls stomatal closure in response to a range of environmental cues (Müller et al., 2017). Stomatal conductance has been demonstrated to be reduced in plants which were nitrogen-limited (Broadley et al., 2001). To demonstrate that stomatal frequency or distribution is involved, Zhu et al. (2020) showed that addition of nitrogen to soil caused significant changes in stomatal pore length and width, and stomatal density.

Another reason that low nitrogen availability can affect photosynthesis is that the enzymes involved in photosynthesis contain nitrogen. The RuBisCO enzyme is responsible for fixing carbon dioxide from the atmosphere for the plant to use in photosynthesis. It is a highly abundant enzyme, making up 30-50% of the soluble protein in leaf material, and for this reason it is widely understood to be the most abundant enzyme in the world (Dey et al., 2015). For this reason, low nitrogen availability directly affects the total RuBisCO content and activity, which is a rate limiting factor in photosynthesis (Kattge et al., 2009).

ATP synthase is the enzyme responsible for catalysing the formation of ATP in cells, which is during photosynthesis in the context of plants. The enzyme uses the electrochemical proton gradient which is generated by the process of photosynthesis to drive the production of ATP. The enzyme contains nitrogen in its structure, in the form of a nitrogenous base, adenine. For this reason, low nitrogen availability in soils can result in reduced abundance of ATP synthase in plants, which subsequently decreases productivity of plants.

Cytochrome b_6f complex is an enzyme involved in the electron transfer chain, and is found in the thylakoid membrane in chloroplasts. This enzyme is involved in the section of the electron transfer chain where electrons are transferred from photosystem II to photosystem I, while simultaneously pumping protons into the thylakoid space. The latter of these two processes contributes to the electrochemical gradient which drives the activity of ATP synthase, which is covered in the previous paragraph. The cytochrome b_6f complex contains a large subunit called cytochrome f, which contains around 2% of the total nitrogen within a

C_3 leaf cell (Evans et al., 2019). The requirement of nitrogen in this structure provides another reason that lack of nitrogen availability can decrease photosynthetic activity.

Photosystem I is one of two photosystems used in the light dependent reactions during photosynthesis. As an integral membrane protein complex, photosystem I is constructed of several protein subunits which all contain nitrogen, including ferredoxin and plastocyanin which are critical for electron transport.

Activity of photosystem II has been shown to be decreased by nitrogen deficiency in wheat (Lu et al., 2001). Similarly to photosystem I, photosystem II is a protein complex consisting of several nitrogen-containing components. These include the D1 and D2 proteins which bind pigments and other cofactors, and the CP43 and CP47 proteins which are chlorophyll-binding and have significant roles in light absorption and energy transfer.

Chlorophyll

Plants tend to allocate approximately 75% of the total leaf nitrogen into chloroplasts to be used in photosynthetic processes (Evans, 1989). Within this amount, a portion of this nitrogen is used within the light-harvesting chlorophyll a/b complex. In both chlorophyll *a* and *b*, their molecular structure contains a central chlorin ring, in which four nitrogen atoms surround a magnesium atom. The requirement of nitrogen as a central part of both chlorophyll *a* and *b* structures provides another clear reason why low nitrogen availability can decrease photosynthetic activity. The decrease in chlorophyll as a result of low nitrogen availability has been demonstrated in the literature (Wu et al., 2019). Plants which have lower chlorophyll content can appear less vibrantly green, and in more severe cases can begin to have a yellow appearance, as the colours of the carotenoids, including the yellow colour of xanthophylls, are no longer masked by the green chlorophyll.

The knock-on effects of insufficient nitrogen are significant, because it is required in so many plant processes throughout the life cycle of the crop affecting photosynthetic capacity. From the effects of reduced canopy expansion and smaller leaf area, to those of lower chlorophyll content and reduced photosynthesis activity, the potential for yield production is impacted throughout by lower assimilate supply to sugar beet roots.

4.1.4 Nitrate reductase

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, and 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 this is the rate-limiting step in the nitrate assimilation process.

Strobilurins have commonly been associated with improved nitrogen metabolism in the literature. It has been suggested that this is due to strobilurins increasing the activity of nitrate reductase, as this enzymes activity has been shown to increase under strobilurin treatment in other crops (Jabs et al., 2004). Nitrate reductase content has been seen to increase when spinach (*Spinacia oleracea*) leaf discs were treated with Kresoxim methyl (a strobilurin fungicide) (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 hypothesised that this is the reason of the resulting increase in nitrate reductase activity and subsequent improved nitrate assimilation.

This effect of increased nitrate reductase activity is also seen with artificial application of hydrogen peroxide (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. These changes in nitrogen metabolism could be involved in the stay-green effect noticed in many strobilurin-treated crops, as senescence timing can be influenced by nitrogen utilisation efficiency (Mirosavljević et al., 2020).

The present study investigated SDHI treatments as well as strobilurins. Although their modes of action are different, they both interact with the pathway of ATP synthesis (strobilurins inhibiting complex III, SDHIs inhibiting complex II), so some of their subsequent interactions with the cell metabolism may be similar. This ATP pathway is the one involved with strobilurins effects on nitrate reductase, so it is possible that SDHIs may have a similar effect.

Aims

- I. To investigate the effects of a range of fungicides on sugar beet's response to different nitrogen availability levels.
  - a. Do sugar beet crops tolerate lower doses of N better if they have received particular fungicides?
  - b. If improved canopy growth is seen under fungicide treatments in low N conditions, does this translate to improved photosynthesis and sugar yields?

Objectives

- I. Compare the physiological responses of plants treated with either an **azole**, a **strobilurin**, an **SDHI**, an **SDHI + azole** combination, a **strobilurin + azole** combination, or left **untreated** under different rates of N application.
  - a. Compare physiological traits such as leaf photosynthesis rate, canopy greenness, canopy cover %, and a range of canopy spectral reflectance indices.
  - b. Compare biomass production and N uptake using growth analysis.
  - c. Compare sugar yields at full machinery harvest.

4.2. Materials and methods

4.2.1 Polytunnel experiment

4.2.1.1 experimental design

The experiment had a randomised block design, with four replicates of each treatment combination.

The variety used was Kortessa (KWS), which has low susceptibility to both powdery mildew and rust compared to others on the BBRO recommended list. Seeds were sown on the 16th March 2022.

Nitrogen treatments:

1. 0 kg N/ha equivalent applied
2. 120 kg N/ha equivalent applied

The soil in the polytunnel boxes was already high in N, as it was sourced from freshly turned topsoil (151 kg N/ha in a 30cm depth profile), so the low N application was reduced to zero to attempt to widen the gap between the two N treatments.

Fungicide treatments were as follows:

0. No fungicide
1. Strobilurin (Pyraclostrobin 200 g/l, 1 l/ha)
2. SDHI (Xemium 62.5 g/l, 1 l/ha)
3. Azole + SDHI (Revysol 100 g/l + Xemium 47 g/l, 1 l/ha)

Fungicide-treated plants were treated three times, with the same fungicide each time, on the 10th June, 8th July, and 1st August 2022. In the high N treatment, nitrogen as ammonium nitrate was applied in a solution on three separate occasions, to reach the equivalent of 120 kg N/ha.

An overview of the treatments in this experiment is displayed below in **Figure 4.3**.

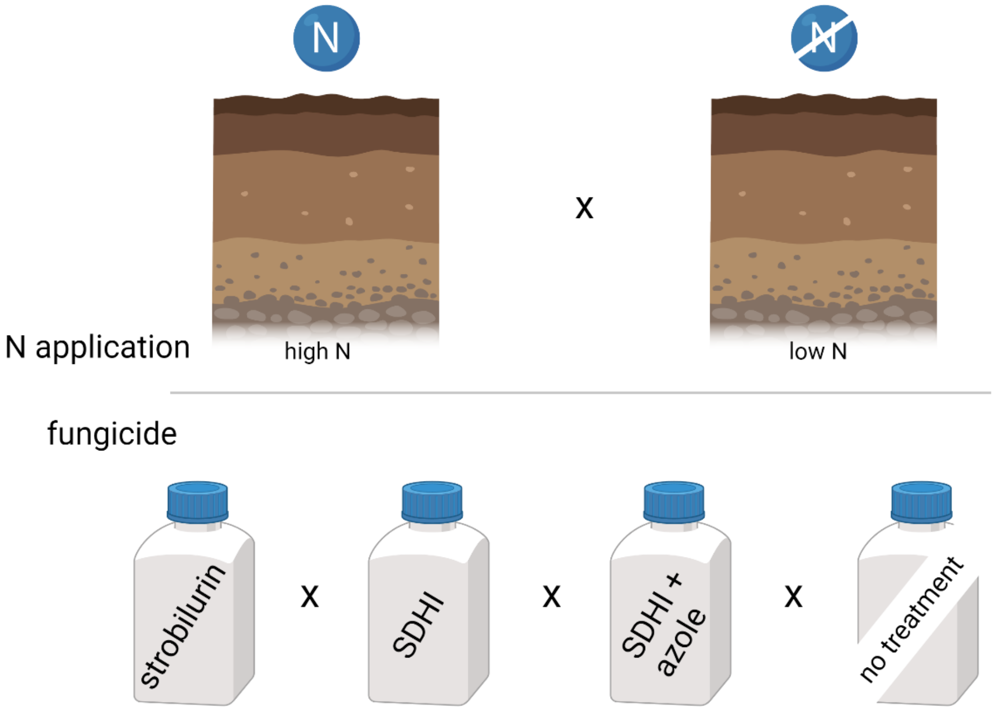

Figure 4.3 Overview of the treatments used in the 2022 polytunnel experiment.

The experiment had a randomised block design, with four replications of each treatment combination. Each box contained 12 plants, arranged in the format of 4 columns and 3 rows, with the centre two plants being used for all measurements unless stated otherwise. The row and plant spacing were the same as the polytunnel experiment in **Chapter 2**.

4.2.1.2 Leaf chlorophyll content (SPAD)

A Minolta SPAD chlorophyll meter (Minolta, Japan) was used to measure leaf chlorophyll content approximately once per two weeks, using the newest fully expanded leaf from each of the central two plants in each box.

4.2.1.3 Reflectance indices

The ASD Fieldspec was used, using the leaf clip function to collect leaf spectral reflectance data, using the newest fully expanded leaf from each of the central two plants in each box. The Fieldspec was out of order after the end of June, so data collection was limited to this early part of the experiment.

4.2.1.4 Harvest

Plants were harvested on 19th October 2022. From each box, the two central plants were collected for further analysis in the laboratory, including top dry weight, root dry weight, and leaf area, while the outer 10 plants were topped, and the roots sent to BBRO for sugar % and impurity analysis.

4.2.1.5 Data analysis

Analysis of variance (ANOVA) suitable for the experiment design was carried out using Genstat 22nd edition, assuming fungicide treatment and nitrogen application to be fixed effects, and replicates to be random effects 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 at assessments over several weeks, a repeated measures ANOVA was used to detect patterns over time. Graphs were developed in Microsoft Excel, using values of the least significant difference of the means as error bars.

4.2.2. Field experiment

4.2.2.1 Experimental design

In the 2023 field experiment, the variety BTS1915 was used. Row spacing was 50cm between each row. The seeds were drilled on 18th May 2023.

Field information for the 2023 crop is as follows:

- Latitude and longitude of experiment: 52.833806, -1.243631
- SNS N Index: 18kgN/ha, SNS Index 0
- Pre-sowing soil nutrient Index: P:4, K:2-, Mg:3, pH:6.7
- Fertiliser: As this experiment focused on nitrogen, this is outlined separately below.
- Herbicides were applied as needed to control weeds

Historic weather data (from Met Office – Sutton Bonington station) for 2023:

Year	**Month**	**Max temp (tmax degC)**	**Rain (mm)**
2023	January	8.5	47.6
2023	February	10.3	6.6
2023	March	10.4	88.6
2023	April	12.9	65.4
2023	May	17.1	43.4
2023	June	22	90.8
2023	July	20.6	82.4
2023	August	21.2	49.6
2023	September	21.6	85.2
2023	October	15.8	124.6
2023	November	10.4	56.8
2023	December	9.4	111

Fungicide treatments:

1. No fungicide
2. Azole + SDHI (Revysol 100 g/l + Xemium 47 g/l, 1 l/ha)
3. Azole + strobilurin (Difenoconazole 125 g/l + Azoxystrobin 125 g/l, 1 l/ha)
4. Azole (Revysol 112.5 g/l, 1.5 l/ha)

Nitrogen treatments:

1. 0 kg N/ha
2. 40 kg N/ha applied to seedbed
3. 40 kg N/ha applied to seedbed, 60 kg N/ha applied at full emergence (100 kg N/ha total)

The initial amount of soil N to 90 cm soil depth from samples taken before drilling was 18 kg N/ha available in the soil before any applications.

The azole + strobilurin mix used in 2023 is the same as the 'treatment 5: Azole + Strobilurin **B**' which was discussed in **chapter 3** of this thesis. This treatment was included in the 2022 field experiment in order to provide a benchmark alongside the other azole + strobilurin mix (**A**) (Cyproconazole + Trifloxystrobin) which has now been removed from the market. Therefore, from the 2023 experiments onwards, any reference to the 'azole + strobilurin' treatment is for the Difenoconazole + Azoxystrobin mix only.

Plants were treated with fungicides twice, when disease first appeared (7th August 2023) and then when disease continued to progress (13th September 2023). A third application was planned if disease was severe later in the season, but this was not required. Nitrogen was first applied to the seed bed, then the second application was made at full emergence.

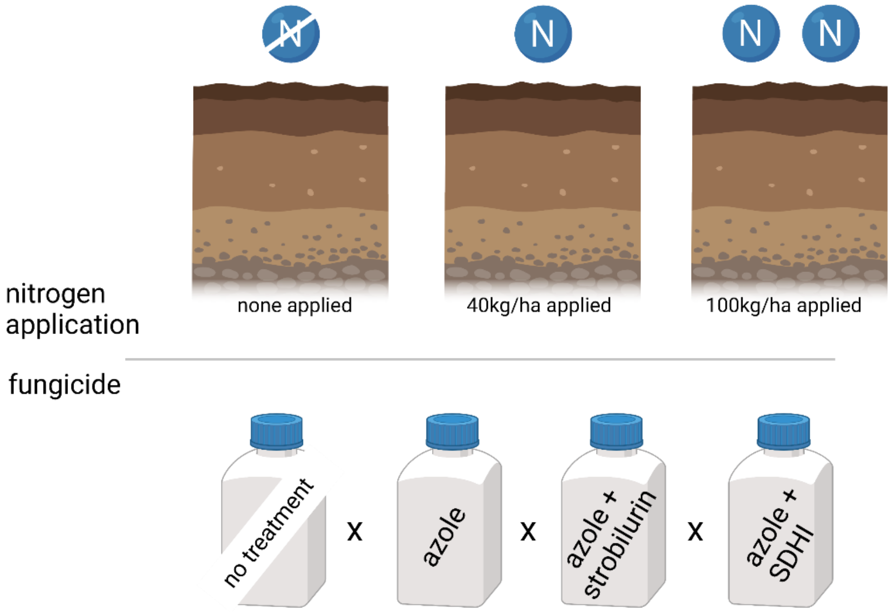

Figure 4.4 Overview of the 2023 field trial, where three nitrogen application levels and four fungicide treatments were included.

The experiment had a split plot design with nitrogen levels randomly assigned on the main plots, and fungicide treatments randomised in the sub-plots. Plots were double drilled, then thinned to approximately 100,000 plants/ha once the plants had fully emerged. Individual plots were 7.5m in length and 3m in width. Each plot was duplicated so that the first 3 m could be used for physiological measurements and final harvest, while the second 3m could be used for biomass harvests without affecting the other measurements. There were discards between each N level.

4.2.2.2 Canopy cover

Photographs were taken at least once per month, depending on weather, 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 47 255). Due to differing weather and lighting conditions, if these set thresholds did not provide full coverage of green area on photos, these were adjusted to match the first image of each block.

4.2.2.3 Canopy spectral reflectance

The ASD Fieldspec was used once at the beginning of the experiment, after nitrogen treatment but before fungicide treatments, using the leaf clip function to collect leaf specific reflectance data. However, the Fieldspec was unavailable due to repairs for the remainder of this experiment.

Reflectance data were collected using a Crop Circle canopy sensor kit (Holland Scientific, Lincoln, Nebraska, USA) attached to a tractor driving alongside the plots. This data was collected approximately once per two weeks.

4.2.2.4 Disease scoring

Disease scoring was carried out during the summer and autumn, to quantify the incidence of rust, powdery mildew and cercospora in the plots. Estimates of the % leaf cover of each disease were observed by walking through each plot and including three central rows.

4.2.2.5 Biomass harvests

Two hand harvests took place; a harvest soon after the first fungicide application (22nd August 2023), then a final hand harvest in December (5th December 2023). In each case, a defined area was harvested (3 rows of 1.5m in August and 2 rows of 1.5m in December), and their biomass measurements were taken both fresh and dry (after drying at 70°C for approximately 1 week). The main focus of the hand harvests was the partitioning of growth, and both above-ground and roots dry weights were recorded.

The full machinery harvest took place in February, in which the plots were harvested and transported to the tarehouse for full yield and sugar analysis. Three central rows were taken to avoid edge effects. At the tarehouse, roots were measured for clean weight, sugar % and impurity content.

4.2.2.6 Statistical analysis

Analysis of variance (ANOVA) for the split-plot experimental design was carried out using Genstat 22nd edition, using nitrogen levels as the main plots and fungicide treatments as the sub-plots. 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 over several weeks, a repeated measures ANOVA was used to detect patterns over time. Graphs were developed in Microsoft Excel, using values of the least significant difference of the means as error bars.

4.2.3. Glasshouse experiment

4.2.3.1 Experimental design

To match the field experiment from 2023, the variety BTS1915 was used in the 2024 glasshouse experiment. Kettering loam soil was used in 5L pots, with approximately 20kg/ha of available nitrogen prior to any fertiliser additions.

Fungicide treatments:

1. No fungicide
2. Azole + SDHI (Revysol 100 g/l + Xemium 47 g/l, 1 l/ha)
3. Azole + strobilurin (Difenoconazole 125 g/l + Azoxystrobin 125 g/l, 1 l/ha)

Nitrogen treatments:

1. 20 kg N/ha equivalent
2. 100 kg N/ha equivalent (40 kg N/ha equivalent applied 1 week after sowing, 60 kg N/ha applied 6 weeks afterward)

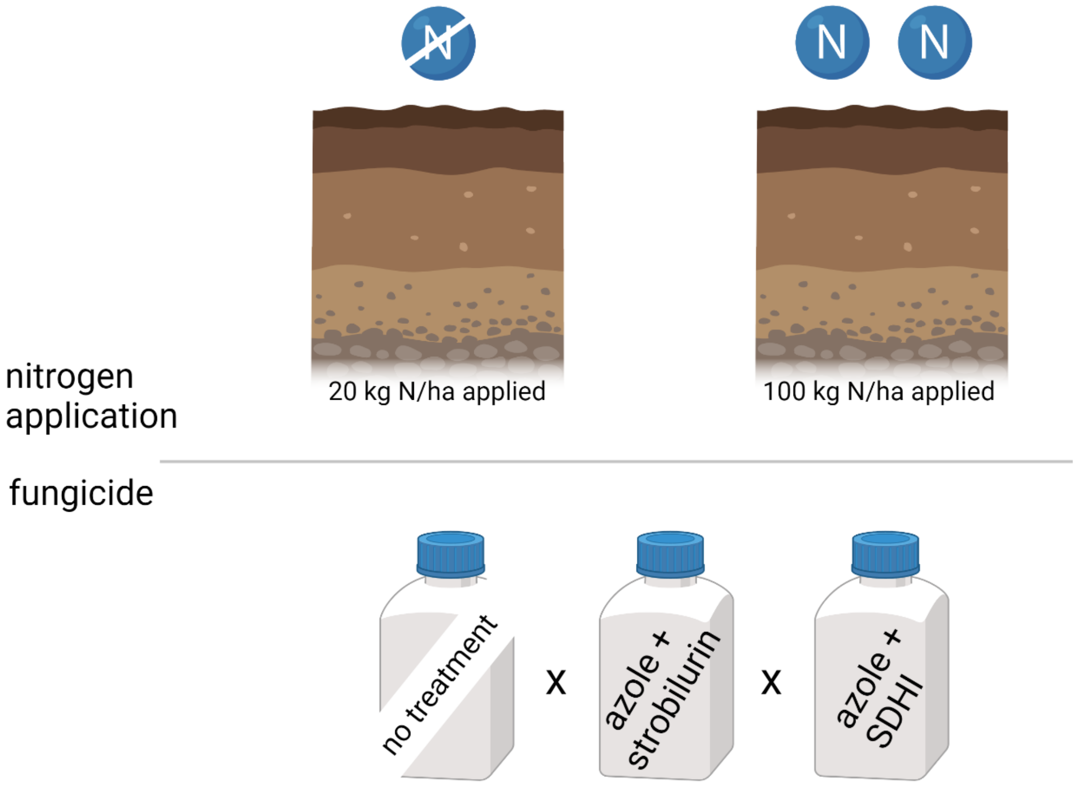

Figure 4.5 Overview of the treatments applied in the 2024 glasshouse experiment.

Layout

The seeds were sown on the 1st May 2024. There was one plant per 5 L pot, and pots were arranged in factorial randomised block design, with four replicates of each treatment combination.

4.2.3.2 Leaf chlorophyll content (SPAD)

A Minolta SPAD chlorophyll meter (Minolta, Japan) was used approximately once per week, using the newest fully expanded leaf from each plant. Three readings were taken per leaf, and a mean calculated.

4.2.3.3 Leaf photosynthetic activity (LI-COR)

A LI-COR LI-6800 (LI-COR Inc, Ne, USA) portable photosynthesis system was used approximately once per week from fungicide application for a total of six weeks. Chlorophyll fluorescence, light-saturated leaf photosynthesis rate and stomatal conductance were the main focus of the analysis. The settings in the cuvette were: temperature 21°C, CO_2 400µmol mol^{-1}, PAR 1200 µmol m^{-2} s^{-1}.

4.2.3.4 Harvest

On the 27th August 2024, the plants were harvested and weighed. Leaf weight, petiole weight and root fresh weight were recorded, and then weighed after drying at 70°C for one week.

4.2.3.5 Data analysis

Analysis of variance (ANOVA) suitable for the fully randomised block experimental design were carried out using Genstat 22nd edition, including nitrogen levels and fungicide treatments as treatments, 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 over several weeks, a repeated measures ANOVA was used to detect patterns over time. Graphs were developed in Microsoft Excel, using values of the least significant differences as error bars.

4.3 Results

4.3.1 Polytunnel experiment

4.3.1.1 Leaf chlorophyll content (SPAD)

Using a repeated measures ANOVA, leaf SPAD was higher ($p = 0.068$) in plants treated with reduced N than those with standard N (**Figure 4.6**), indicating a trend for higher chlorophyll content. Only including the dates after first fungicide application in the analysis, there was no effect of fungicide on leaf SPAD ($p=0.229$).

Figure 4.6 Leaf SPAD values for plants treated with standard nitrogen application and those which received none. P = 0.068 for the differences between nitrogen treatments.

4.3.1.2 Canopy spectral reflectance

Unless otherwise stated, the data displayed in this section is from the assessment on 29th June 2022, which was 19 days after fungicide application. Other dates of data collection were before fungicide application or only just after application, and will be specifically referenced where included.

Blue green index

Blue green index (BGI) was higher for the SDHI and the SDHI + azole treatments than for the no fungicide control (close to significant, p=0.072, **Figure 4.7**).

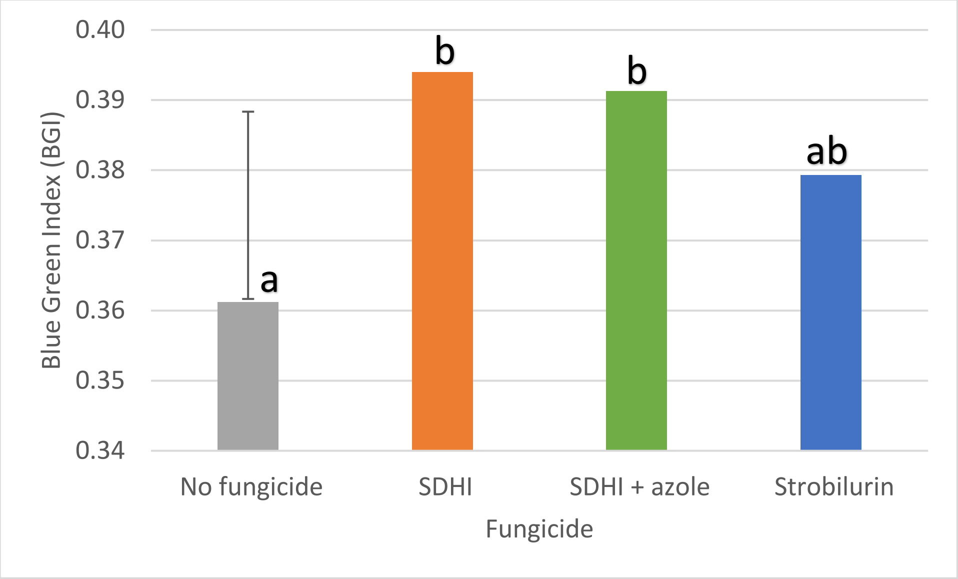

Figure 4.7 Blue Green Index values of plants treated with different fungicides in the polytunnel experiment, p=0.072.

There was no significant interaction between the nitrogen level and the fungicide treatment for BGI (p=0.599, **Figure 4.8**). There was also no significant difference between the two nitrogen levels, p=0.112.

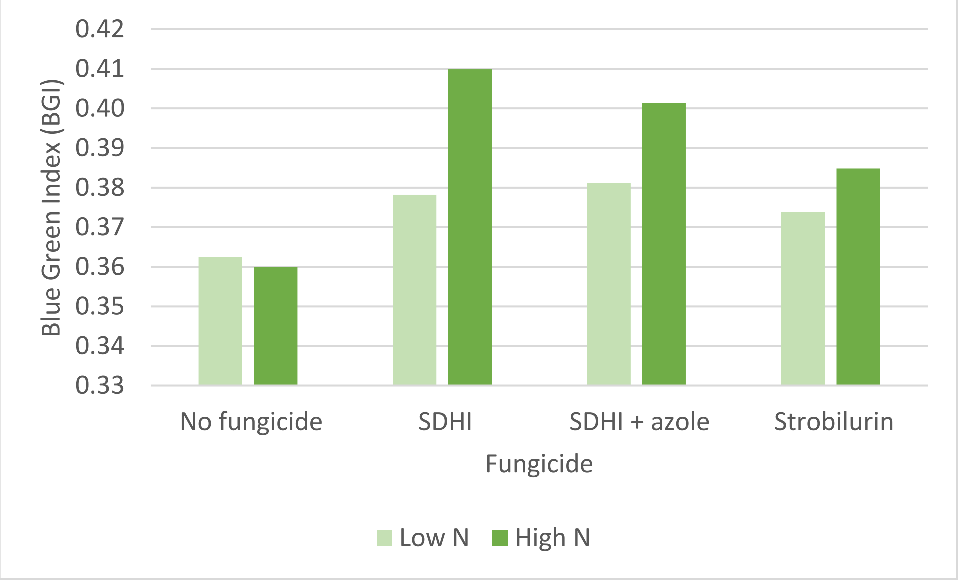

Figure 4.8 Blue Green Index values of plants treated with different nitrogen levels and fungicide treatments in the polytunnel experiment, no interaction found (p=0.599).

Carotenoid reflectance index

Carotenoid reflectance index was higher for the no fungicide control and the strobilurin treatment than for the SDHI + azole treatment (p=0.03, **Figure 4.9**).

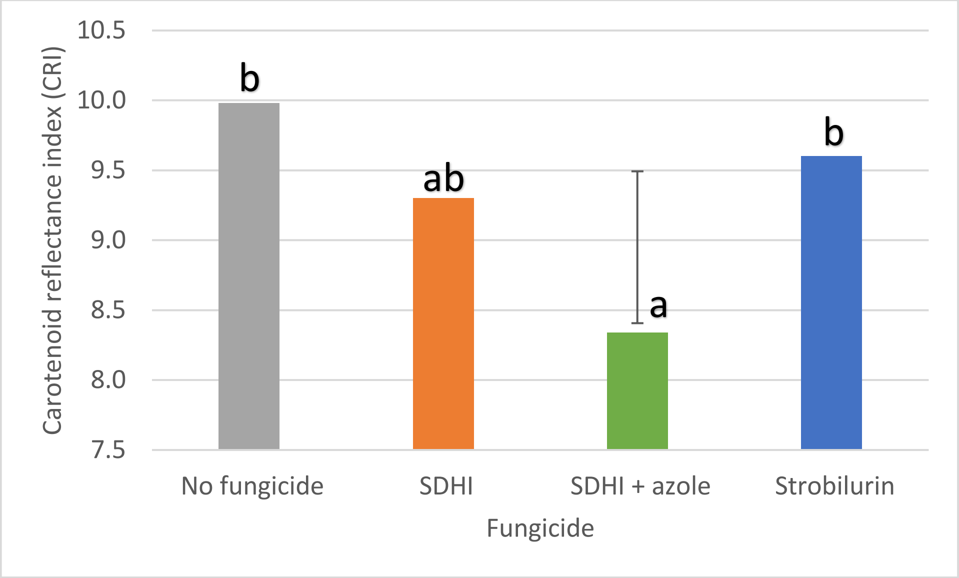

Figure 4.9 Carotenoid Reflectance Index values of plants treated with different fungicides in the polytunnel experiment, p=0.03.

As the Carotenoid Reflectance Index is indicative of the ratio of carotenoid content against chlorophyll content, values tend to be higher in plants which are stressed or lacking nutrients. For this reason, a lower value suggests a healthier plant with lower stress.

There was no significant interaction between the nitrogen level and the fungicide treatment for Carotenoid Reflectance Index (p=0.146, **Figure 4.10**). There was also no significant difference between the two nitrogen levels (p=0.840).

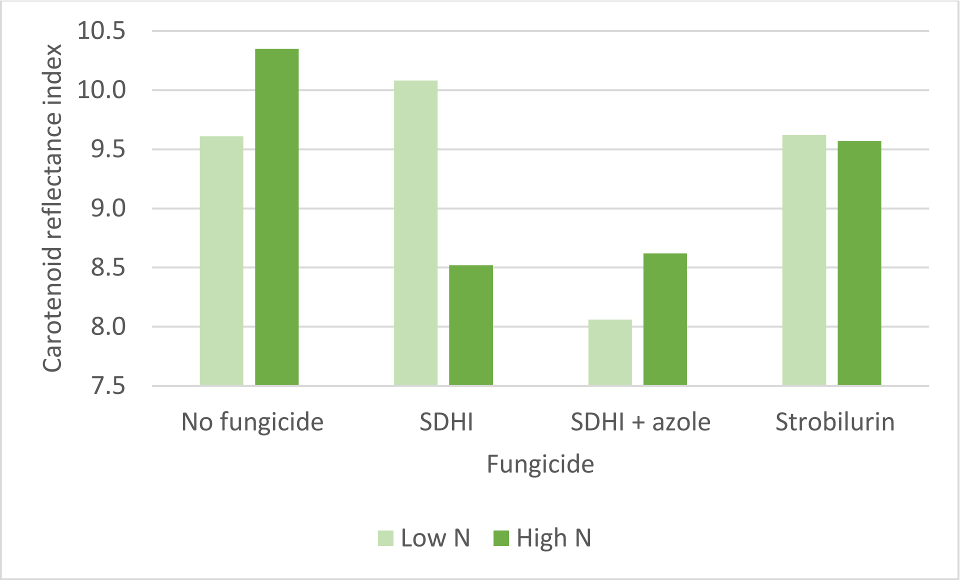

Figure 4.10 Carotenoid Reflectance Index values of plants treated with different nitrogen levels and fungicide treatments in the polytunnel experiment, no interaction found (p=0.146).

NDVI

NDVI was higher in plants treated with the strobilurin and the no fungicide control compared to the SDHI + azole treatment (p=0.012, **Figure 4.11**).

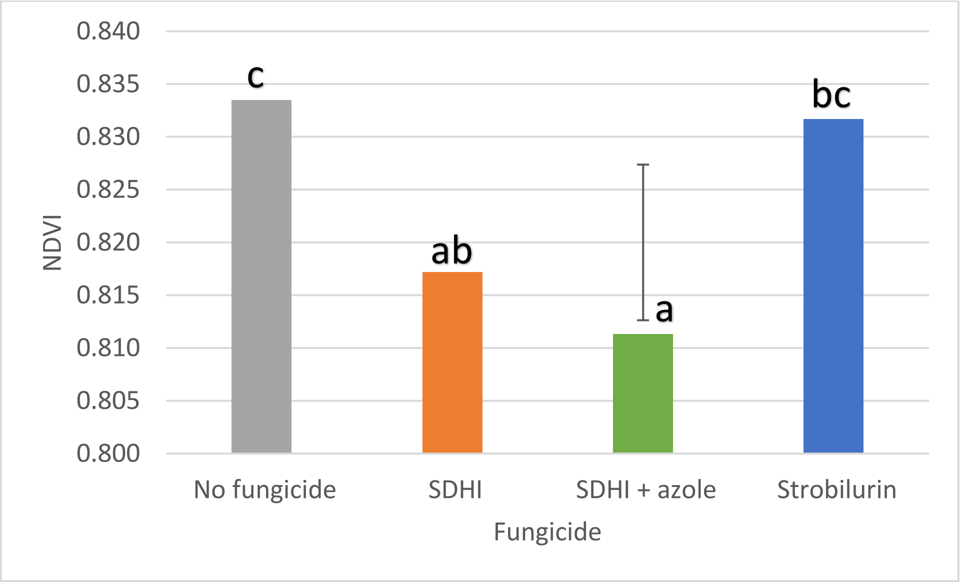

Figure 4.11 NDVI values of plants treated with different fungicides in the polytunnel experiment, p=0.012.

There was no significant interaction between the nitrogen level and the fungicide treatment for NDVI (p=0.293, **Figure 4.12**). There was also no significant difference between the two nitrogen levels (p=0.504).

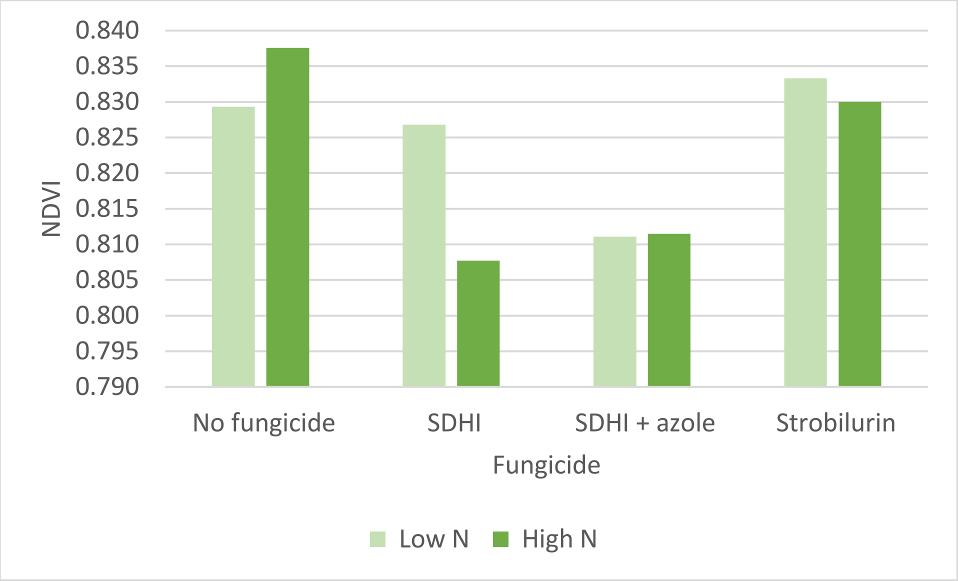

Figure 4.12 NDVI values of plants treated with different nitrogen levels and fungicide treatments in the polytunnel experiment, no interaction found (p=0.293).

NDWI

NDWI was higher in plants treated with the strobilurin and the no fungicide control than in plants treated with the SDHI (p=0.031, **Figure 4.13**).

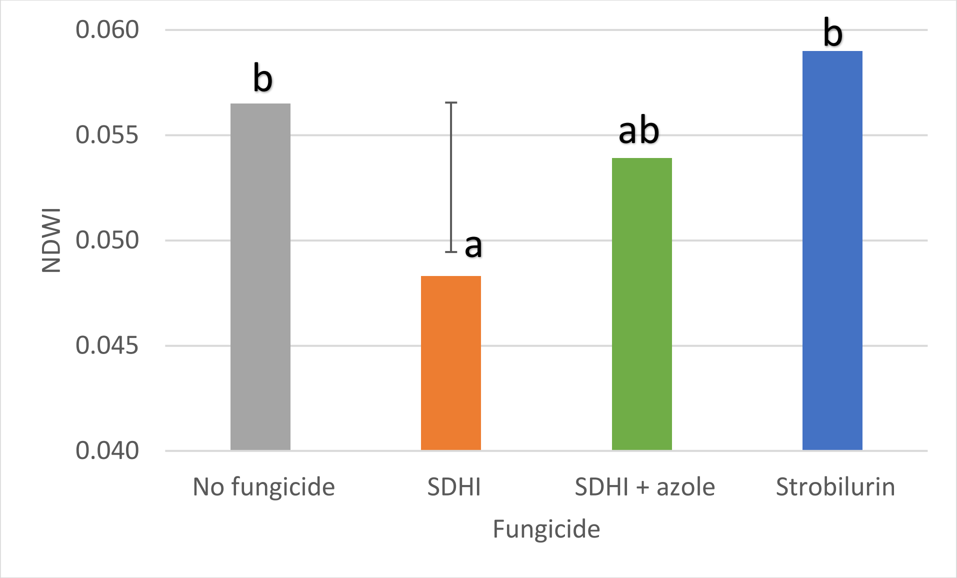

Figure 4.13 NDVI values of plants treated with different fungicides in the polytunnel experiment, p=0.031.

NDWI (Normalised Difference Water Index) decreases in plants with water stress, so a higher value is indicative of a plant with more sufficient water intake. Plants with higher nitrogen content can be associated with a lower value in many water indices (Govind et al., 2005), so these NDWI results could suggest that nitrogen may have been more abundant in the plants treated with the SDHI than the no fungicide control and the strobilurin treatment.

There was no significant interaction between the nitrogen level and the fungicide treatment for NDWI (p=0.394, **Figure 4.14**). There was also no significant difference between the two nitrogen levels (p=0.859).

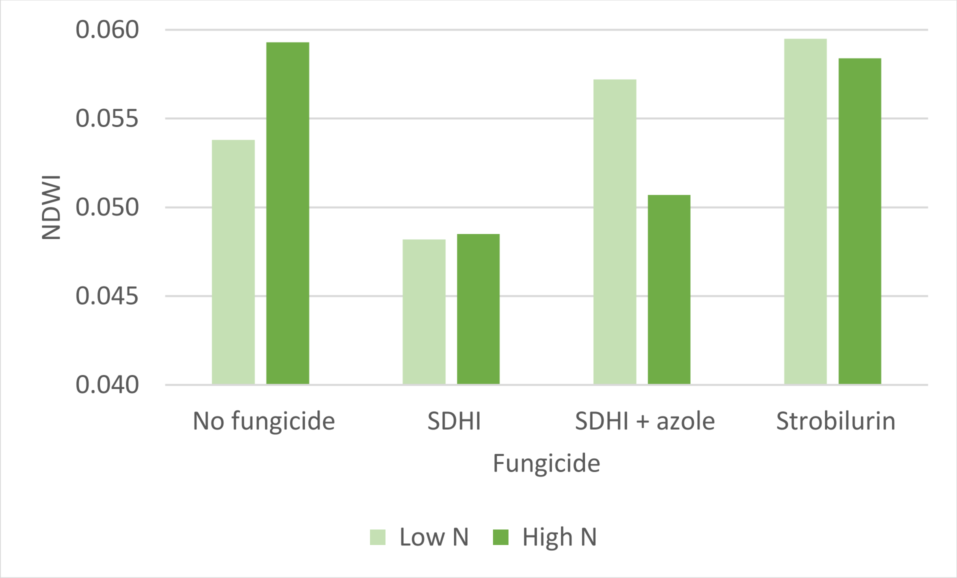

Figure 4.14 NDWI values of plants treated with different nitrogen levels and fungicide treatments in the polytunnel experiment, no interaction found (p=0.394).

NPCI

NPCI (Normalised Pigment Chlorophyll ratio Index) was higher in the plants treated with the SDHI and the SDHI + azole treatments than in the plants treated with the strobilurin (almost significant, p=0.052, **Figure 4.15**).

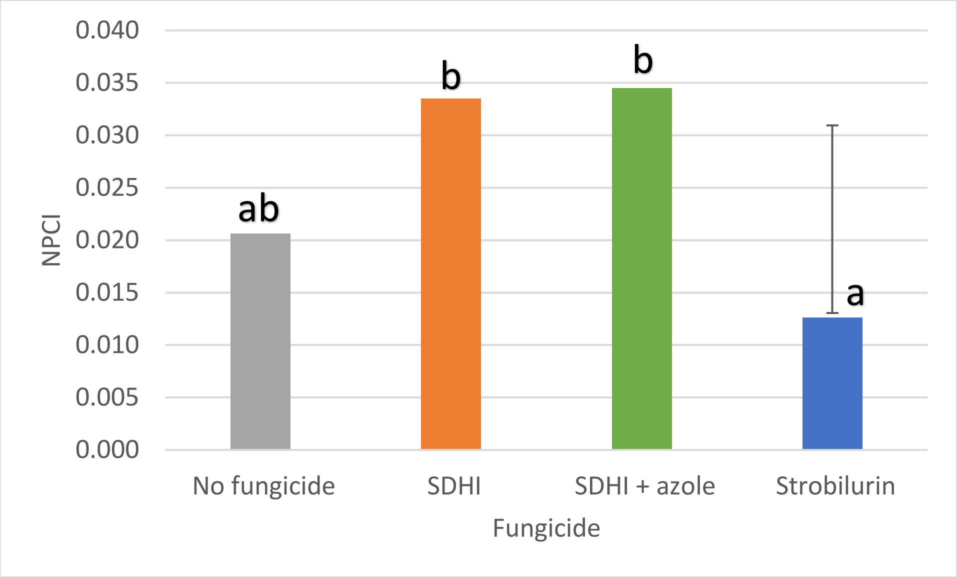

Figure 4.15 NPCI values of plants treated with different fungicides in the polytunnel experiment, p=0.052.

This reflectance index aims to compare the presence of chlorophyll compared with other pigments (namely the carotenoids). These results are similar to the inverse of the Carotenoid Reflectance Index, further suggesting that the ratio of chlorophyll to other pigments was better optimised in the SDHI and the SDHI + azole treatment than the strobilurin treatment.

There was no significant interaction between the nitrogen level and the fungicide treatment for NPCI (p=0.901, **Figure 4.16**). There was also no significant difference between the two nitrogen levels (p=0.686).

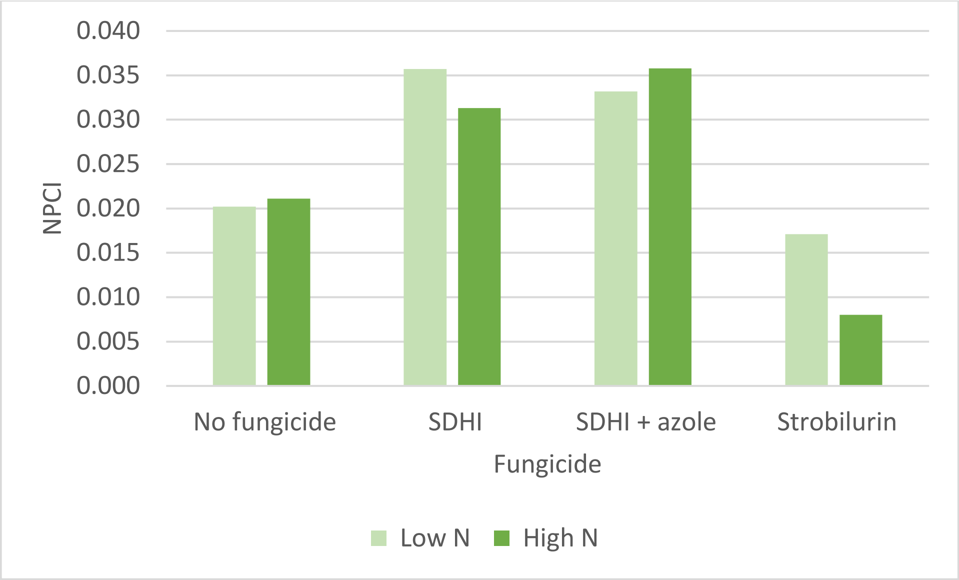

Figure 4.16 NPCI values of plants treated with different nitrogen levels and fungicide treatments in the polytunnel experiment, no interaction found (p=0.901).

RARSb

RARSb was higher in the strobilurin and the no fungicide control than in the SDHI + azole treatment (nearly statistically significant, p=0.092, **Figure 4.17**).

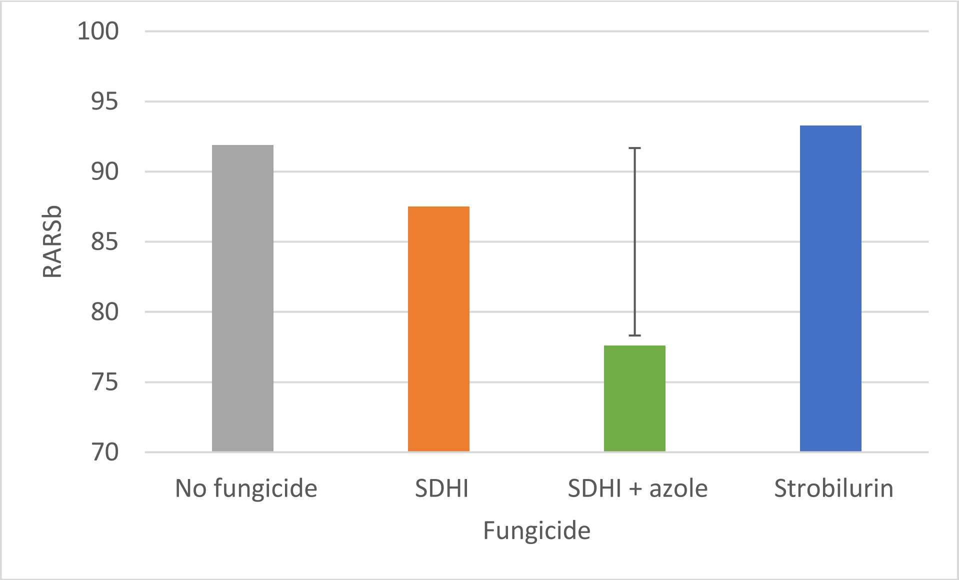

Figure 4.17 RARSb values of plants treated with different fungicides in the polytunnel experiment, p=0.092.

There was no significant interaction between the nitrogen level and the fungicide treatment for RARSb (p=0.164, **Figure 4.18**). There was also no significant difference between the two nitrogen levels (p=0.920).

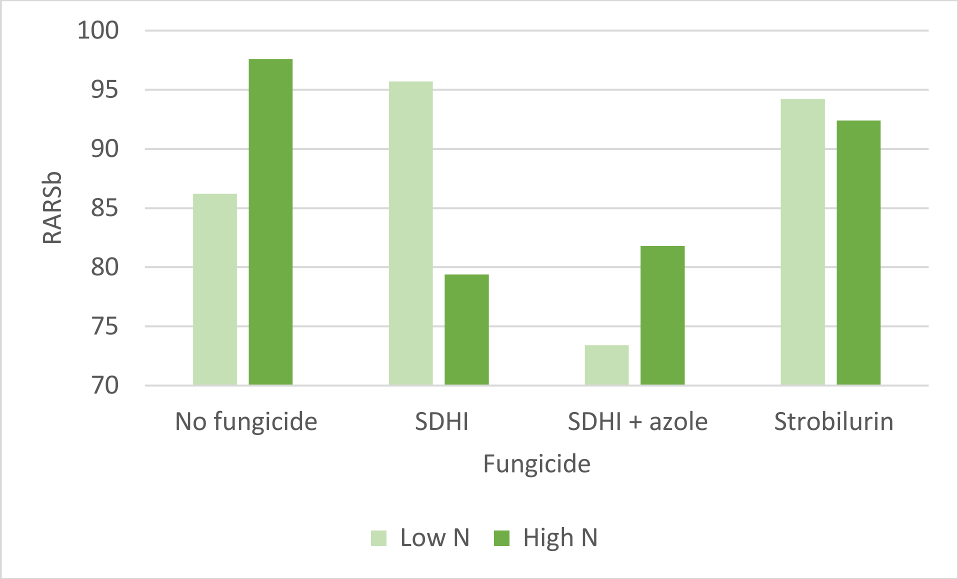

Figure 4.18 RARSb values of plants treated with different nitrogen levels and fungicide treatments in the polytunnel experiment, no interaction found (p=0.164).

4.3.1.3 Harvest assessments

The results revealed few significant differences between treatments. There was a trend for the high nitrogen treatment to produce higher leaf area (p = 0.072, **Figure 4.19**), and a higher leaf dry weight (p = 0.074, **Figure 4.20**). There was no effect of fungicides on leaf area (p=0.901) nor on leaf dry weight (p=0.807).

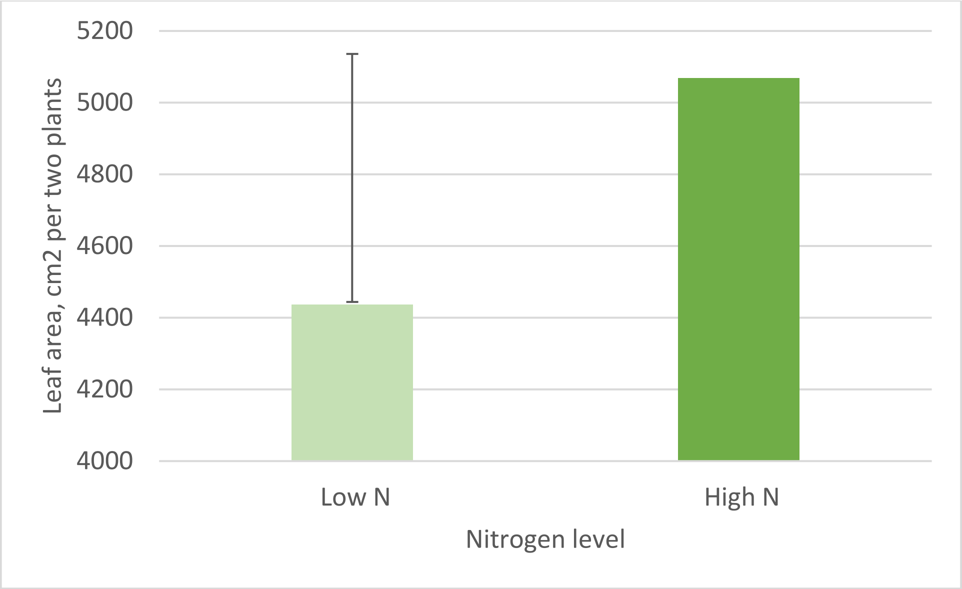

Figure 4.19 Leaf area at harvest for plants which received no nitrogen treatment and those which received a standard dose, p=0.072.

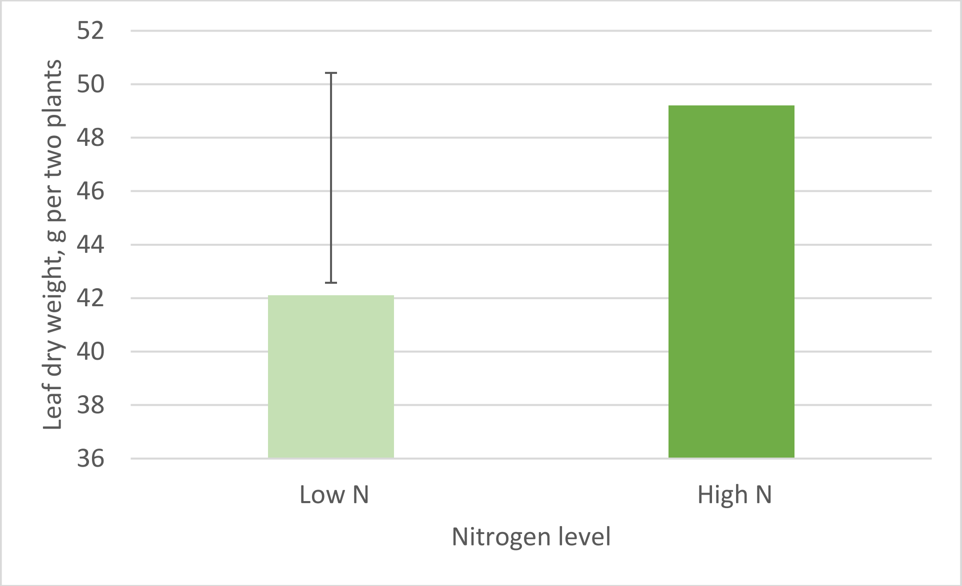

Figure 4.20 Leaf dry weight at harvest for low N and high N plants, p=0.074.

Low N plants had a higher sugar % in the roots than high N plants (p = 0.016, **Figure 4.21**). There was no effect of fungicides on sugar % (p=0.453).

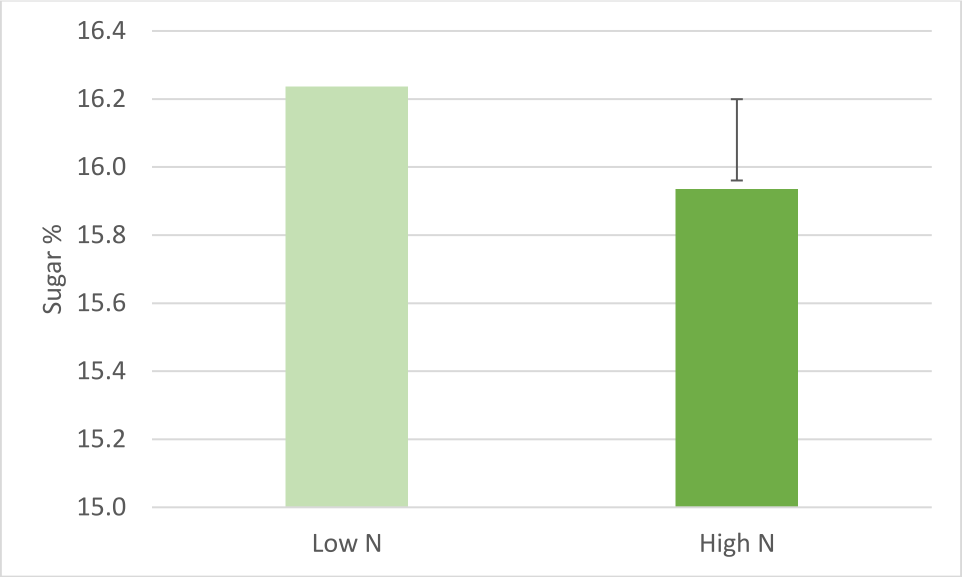

Figure 4.21 Sugar % in the root from plants grown with low N and high N. P = 0.016 for differences between nitrogen treatments.

These data suggests that the increased N plants could have had excess nitrogen, and that this may have caused partitioning issues, where the plant became more productive above ground rather than focusing on storage in the root, and the sugar content suffered as a result.

4.3.2 Field experiment

4.3.2.1 Canopy cover

In the field experiment in 2023, canopy cover % differed between N treatments (**Figure 4.22**, p<0.001 in a repeated measures analysis). This effect was stronger at the start of the season, and became smaller and non-significant as the season went on.

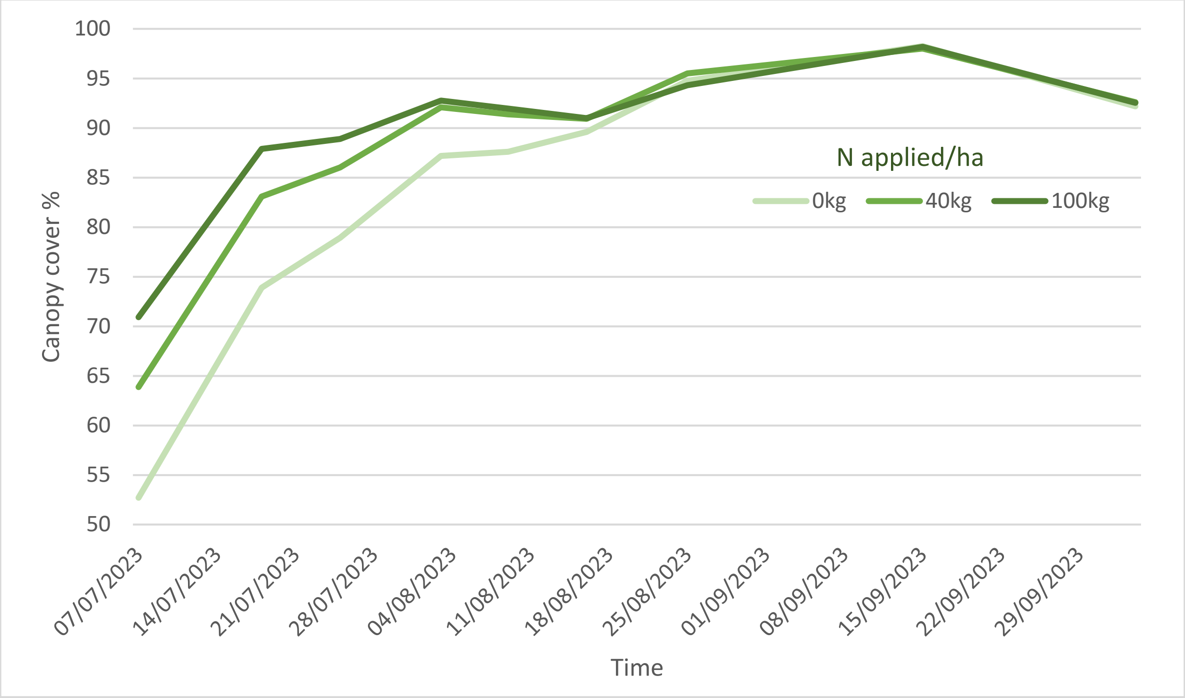

Figure 4.22 Canopy cover % throughout the season for three nitrogen levels (p<0.001 in a repeated measures analysis).

The photographs were taken from directly above the plots, so this may be why the canopy cover % was similar between treatments even though the canopy growth may still have differed between treatments. The high nitrogen plots were visibly taller than the plots which received no nitrogen, but canopy assessed from directly above was similar.

Figure 4.23 shows photographs from the low and high nitrogen treatments as seen in July compared to the photos from September.

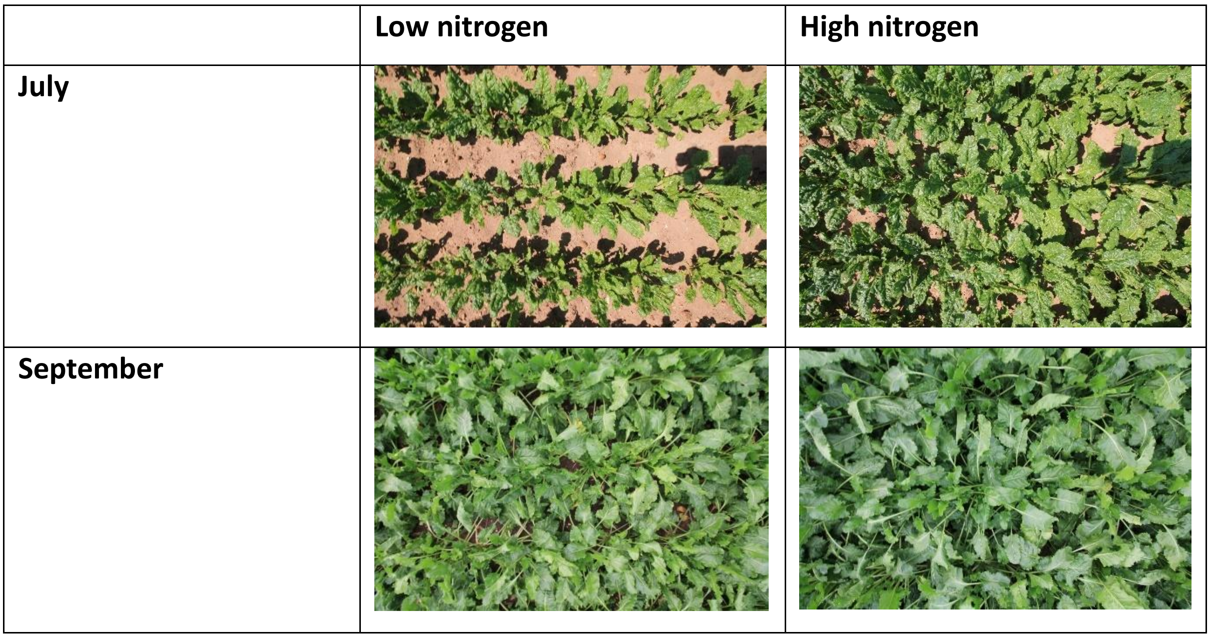

Figure 4.23 Comparison between photographs taken of low and high nitrogen plots in July and September, as a visual addition to the canopy cover % data in Figure 4.22.

4.3.2.2 Canopy spectral reflectance

NDVI

NDVI values are indicative of green area, so a larger value is associated with a higher canopy cover %, and subsequently higher light interception.

There was a highly significant relationship between NDVI and nitrogen treatment in this experiment, with the high nitrogen and medium nitrogen treatments having higher NDVI than the low nitrogen treatment (using a repeated measures analysis, p<0.001, **Figure 4.24**).

treatment	mean	multiple comparisons
100kg	0.8610	b
40kg	0.8570	b
0kg	0.8469	a

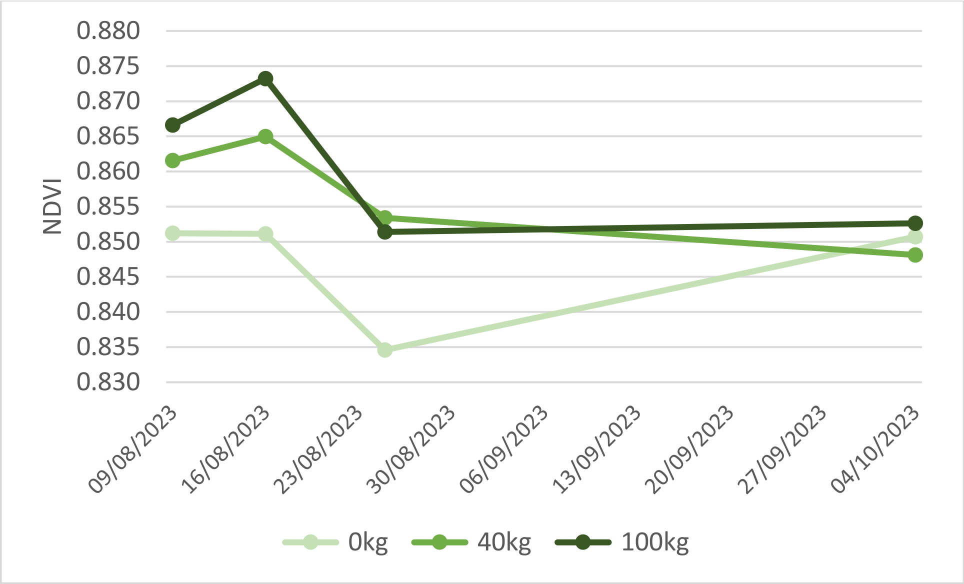

Figure 4.24 NDVI values recorded at canopy level between three different nitrogen treatments in the field experiment, p<0.001. Results from Duncan's multiple range tests included in table within figure to identify the individual differences between treatments.

For NDVI there was an effect of fungicide treatment, with the azole + SDHI treatment having higher NDVI values than the no fungicide control (using a repeated measures analysis, p=0.027, **Figure 4.25**).

treatment	mean	multiple comparisons
Azole + SDHI	0.8593	b
Azole	0.8561	ab
Azole + strobilurin	0.8539	ab
No fungicide	0.8505	a

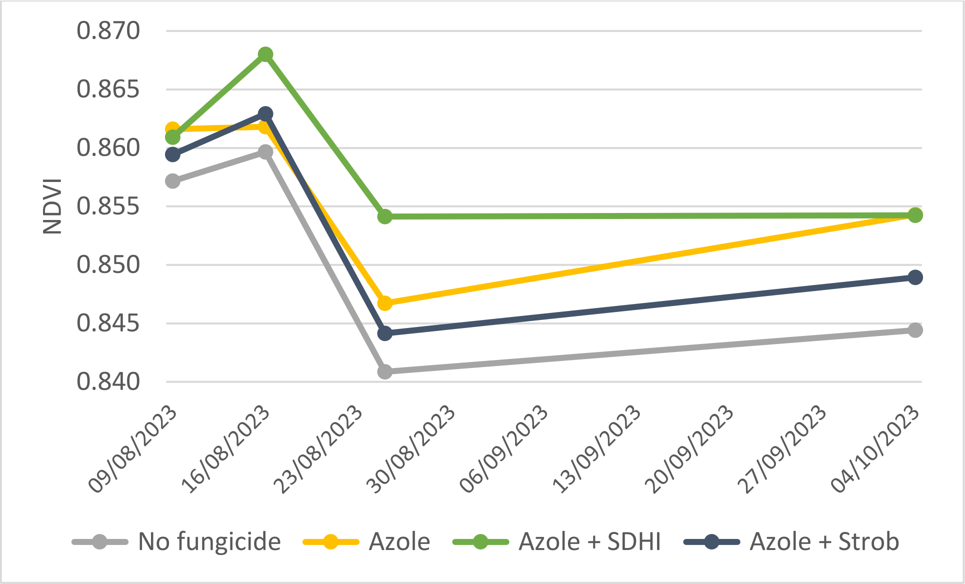

Figure 4.25 NDVI values recorded at canopy level between three different fungicide treatments in the field experiment, p=0.027. Results from Duncan's multiple range tests included in table within figure to identify the individual differences between treatments.

NDRE

NDRE is similar to NDVI, however, by utilising the red-edge part of the electromagnetic spectrum, this value can provide a better insight into plant health and active plant material. Plants might have an adequate green area but with mildly damaged or stressed leaves, which is where NDVI is more likely to record these differences. A high NDRE value is indicative of a large area of *healthy* canopy, and lower values are associated with plants undergoing stress, or with lower chlorophyll content.

There was a highly significant effect of N treatment for NDRE, with differences in the order high N > medium N > low N (using a repeated measures analysis, p<0.001, **Figure 4.26**).

treatment	mean	multiple comparisons
100kg	0.3361	c
40kg	0.3269	b
0kg	0.3196	a

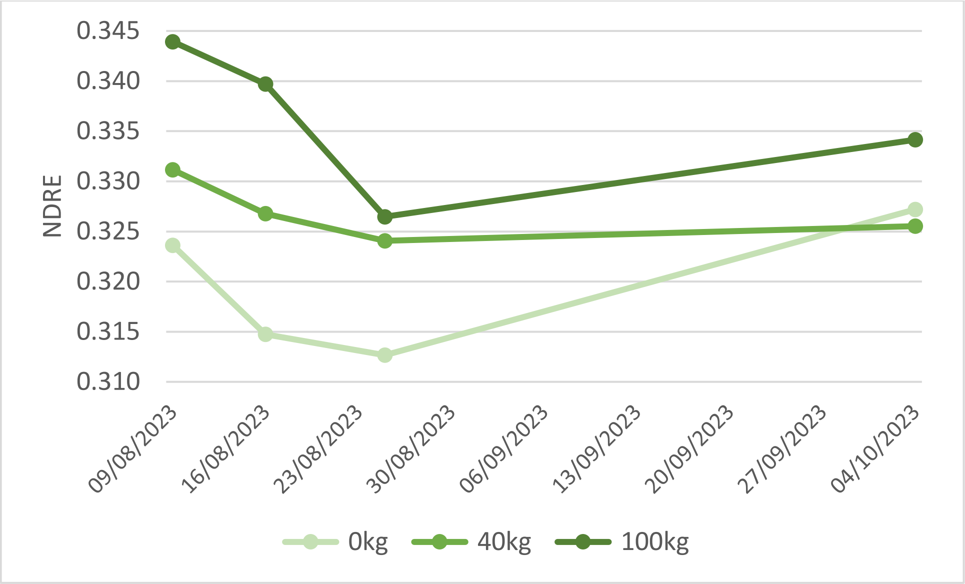

Figure 4.26 NDRE values recorded at canopy level between three different nitrogen treatments in the field experiment, p<0.001. Results from Duncan's multiple range tests included in table within figure to identify the individual differences between treatments.

For NDRE there was an effect of fungicide treatment, with the azole + SDHI treatment having higher NDVI values than the no fungicide control (p=0.031, **Figure 4.27**).

treatment	mean	multiple comparisons
Azole + SDHI	0.3316	b
Azole	0.3288	ab
Azole + strobilurin	0.3260	ab
No fungicide	0.3236	a

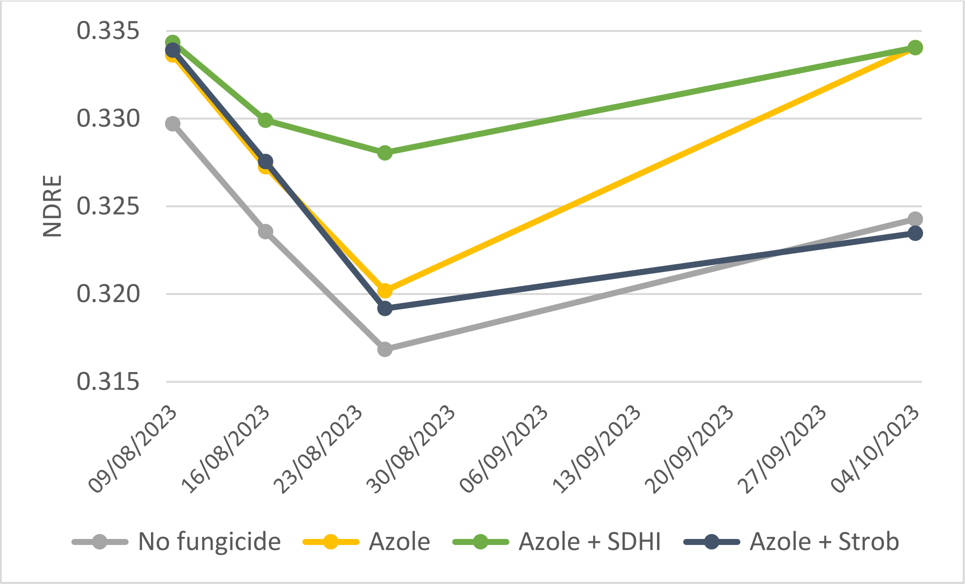

Figure 4.27 NDRE values recorded at canopy level between three different fungicide treatments in the field experiment, p=0.031. Results from Duncan's multiple range tests included in table within figure to identify the individual differences between treatments.

Both NDVI and NDRE were found to be higher in the azole + SDHI treatment than in the no fungicide control, with the other fungicide treatments not being significantly different from each other. There was no interaction between fungicide and nitrogen level for these traits.

4.3.2.3 Disease assessment

Rust

Overall the levels of rust % cover in the experiment were low, reaching a maximum of 5% in the untreated control by the end of October. Rust % cover across the plots was higher in the no fungicide control than in all other fungicide treatments (using a repeated measures analysis, p<0.001, **Figure 4.28**). Excluding the no fungicide control, the other fungicide treatments did not differ from one another, as shown in the table within **Figure 4.28** which includes the results of Duncan's multiple range test for multiple comparisons (note: the graph includes all disease scoring dates, but the multiple comparisons table is calculated excluding the first date as this was before fungicide application).

treatment	mean	multiple comparisons
No fungicide	2.9167	b
Azole + strobilurin	0.3750	a
Azole	0.3083	a
Azole + SDHI	0.2458	a

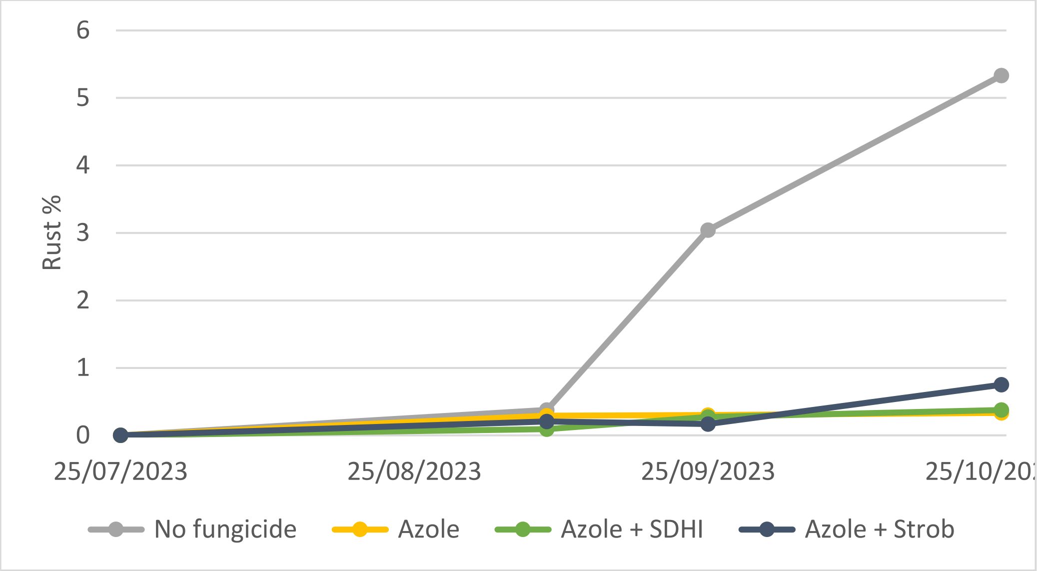

Figure 4.28 Rust % leaf cover for fungicide treatments, p<0.001. Results from Duncan's multiple range tests included in table within figure to identify the individual differences between treatments, including only the dates after the first fungicide treatment.

There was no significant difference in rust % between the nitrogen treatments (repeated measures, p=0.861, **Figure 4.29**).

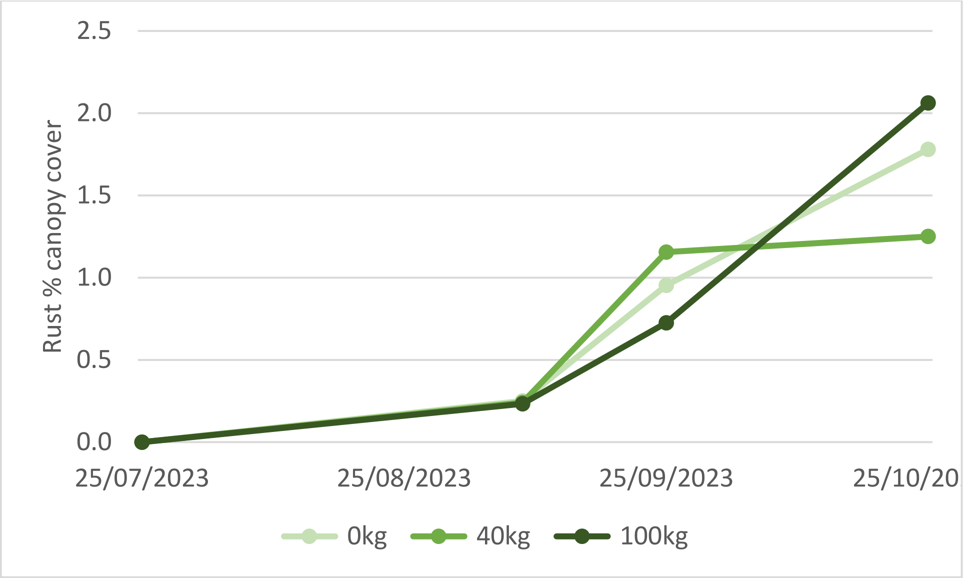

Figure 4.29 Rust % cover across plots between different nitrogen treatments, no significant differences found, p=0.861.

There was no interaction found between the effects of fungicide treatment and nitrogen treatment on rust % (repeated measures analysis using only dates after fungicide treatment, p=0.983, **Figure 4.30**).

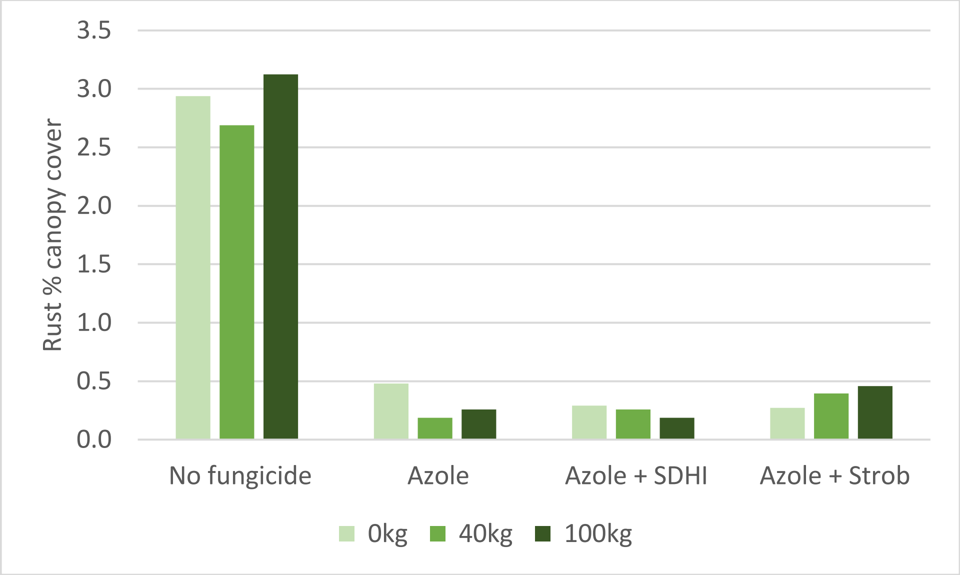

Figure 4.30 Rust % canopy cover between plants treated with different fungicides, with a range of nitrogen levels, no interaction found, p=0.983).

Cercospora

Overall the levels of cercospora % cover in the experiment were relatively low, reaching a maximum of 7% in the untreated control by the end of October. Cercospora % cover across the plots was significantly higher in the no fungicide control than in all other fungicide treatments (using a repeated measures analysis, $p<0.001$, **Figure 4.31**). Excluding the no fungicide control, the other fungicide treatments did not differ, as shown in the table within **Figure 4.31** which includes the results of Duncan's multiple range test for multiple comparisons (note: the graph includes all disease scoring dates, but the multiple comparisons table is calculated excluding the first date as this was before fungicide application).

treatment	mean	multiple comparisons
No fungicide	3.475	b
Azole + strobilurin	1.792	a
Azole	1.214	a
Azole + SDHI	1.179	a

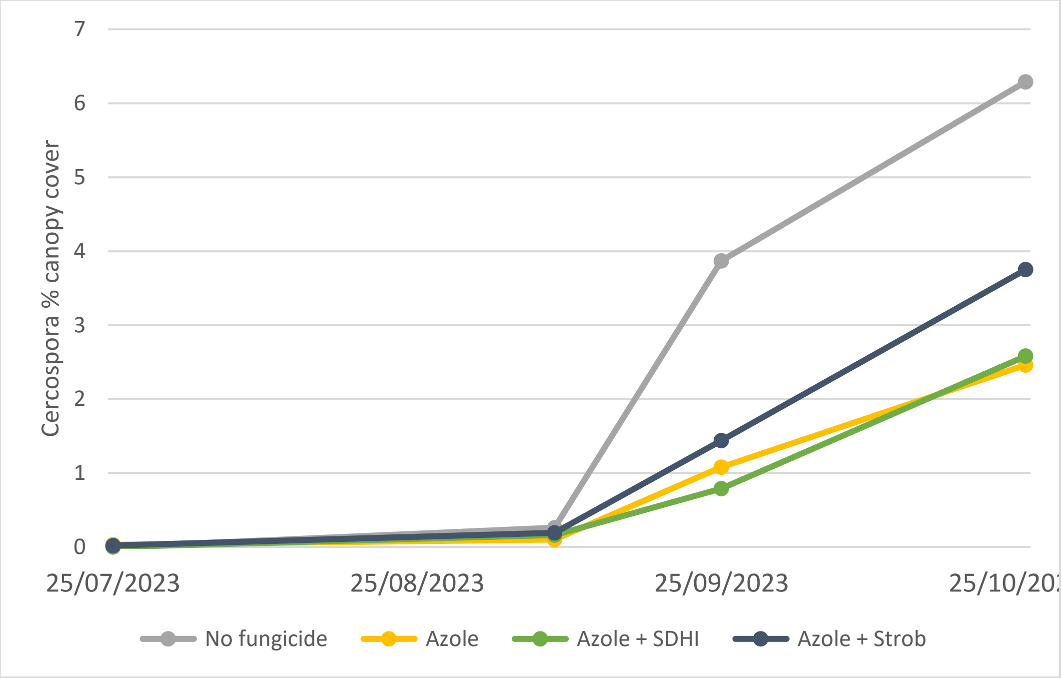

Figure 4.31 Cercospora % cover across plots between different fungicide treatments, $p<0.001$. Results from Duncan's multiple range tests included in table within figure to identify the individual differences between treatments, including only the dates after the first fungicide treatment.

There was no effect of nitrogen level on cercospora % canopy cover (p=0.109, **Figure 4.32**) based on the repeated measures analysis.

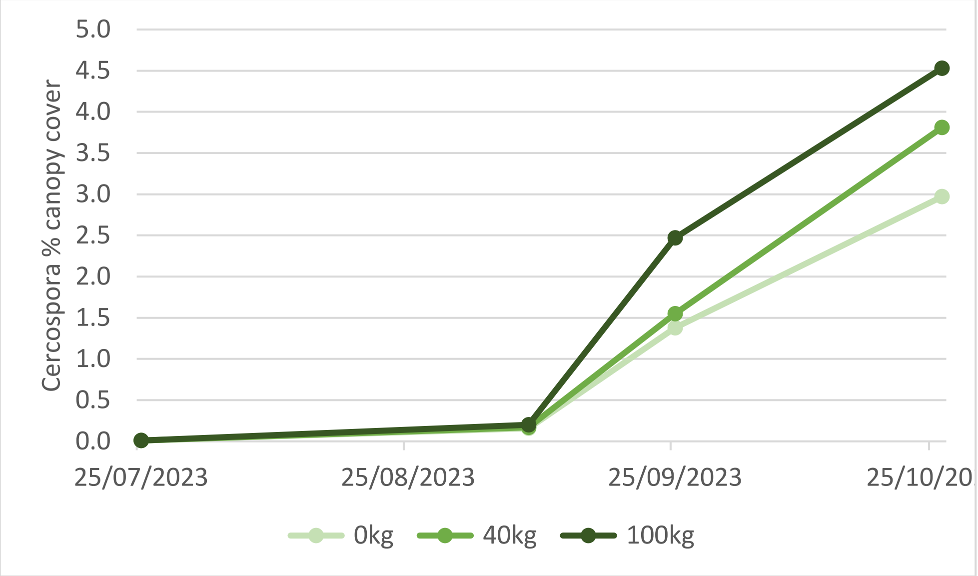

Figure 4.32 Cercospora % canopy cover of plants treated with different doses of nitrogen, almost statistically significant, p=0.109).

There was no interaction found between the effects of fungicide treatment and nitrogen treatment on cercospora % (repeated measures analysis using only dates after fungicide treatment, p=0.817, **Figure 4.33**).

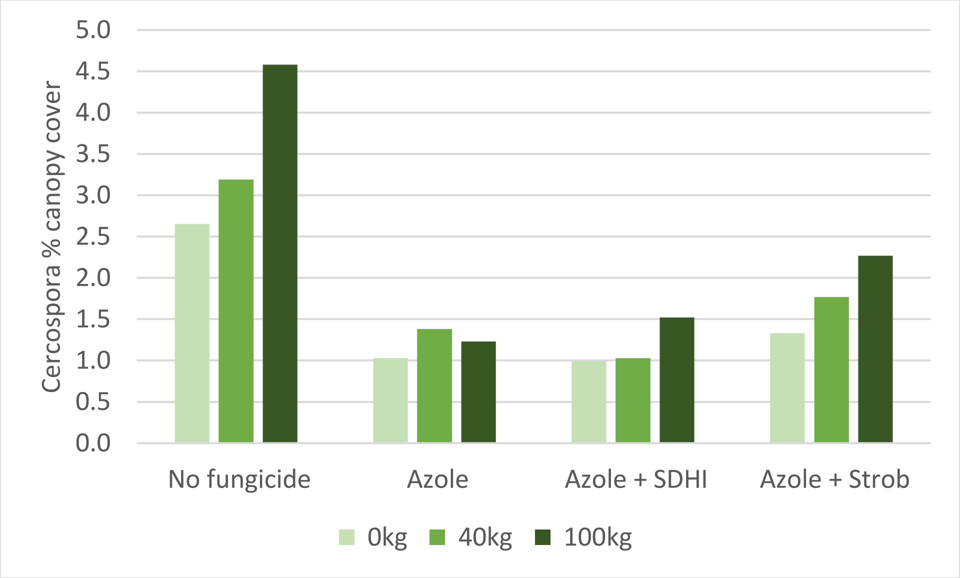

Figure 4.33 Cercospora % canopy cover between plants treated with different fungicides, with a range of nitrogen levels, no interaction found, p=0.817.

Powdery mildew

Powdery mildew generally had very low incidence throughout this experiment, appearing and disappearing again by the final disease scoring date. A repeated measures analysis showed that the no fungicide control had higher powdery mildew % cover on the canopy than all other treatments (p<0.001, **Figure 4.34**) (note: the graph includes all disease scoring dates, but the multiple comparisons table is calculated excluding the first date as this was before fungicide application).

treatment	mean	multiple comparisons
No fungicide	3.944	b
Azole	0.139	a
Azole & strobilurin	0.000	a
Azole + SDHI	0.000	a

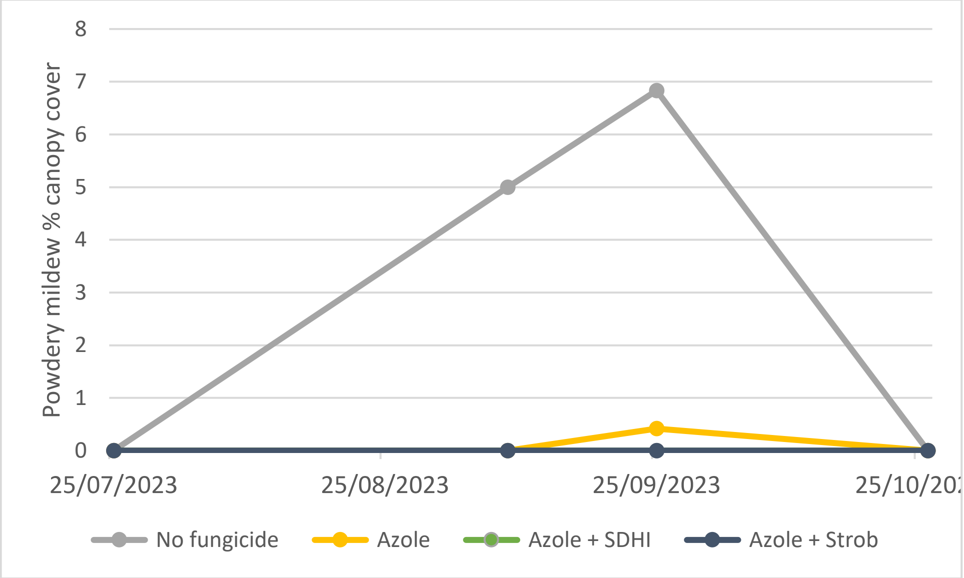

Figure 4.34 Powdery mildew % cover across plots between different fungicide treatments, p<0.001. Results from Duncan's multiple range tests included in table within figure to identify the individual differences between treatments, including only the dates after the first fungicide treatment.

There was no significant difference in powdery mildew % between the different nitrogen treatments (repeated measures, p=0.410, **Figure 4.35**).

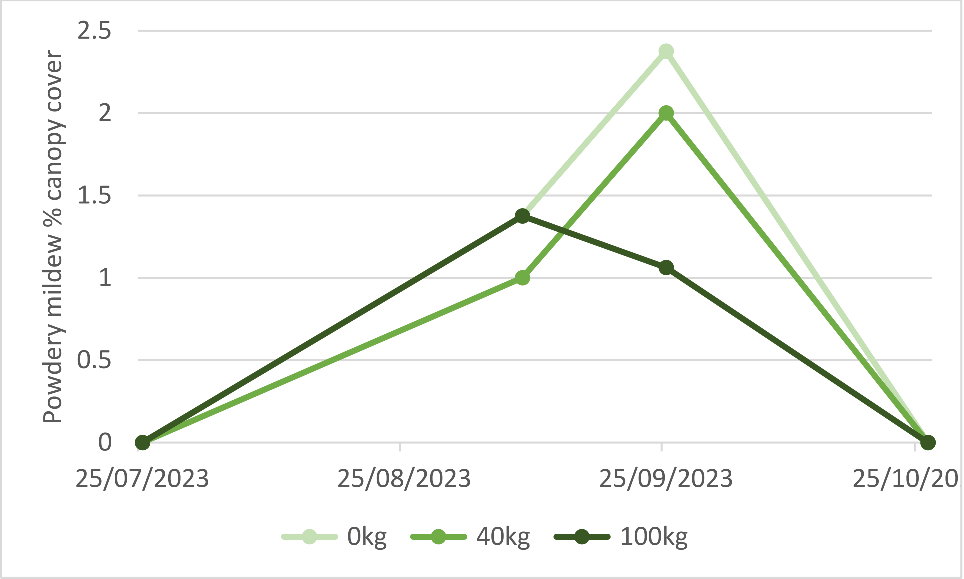

Figure 4.35 Powdery mildew % cover across plots between different nitrogen treatments, no significant differences found, p=0.410.

There was no interaction found between the effects of fungicide treatment and nitrogen treatment on powdery mildew % (repeated measures analysis using only dates after fungicide treatment, p=0.353, **Figure 4.36**).

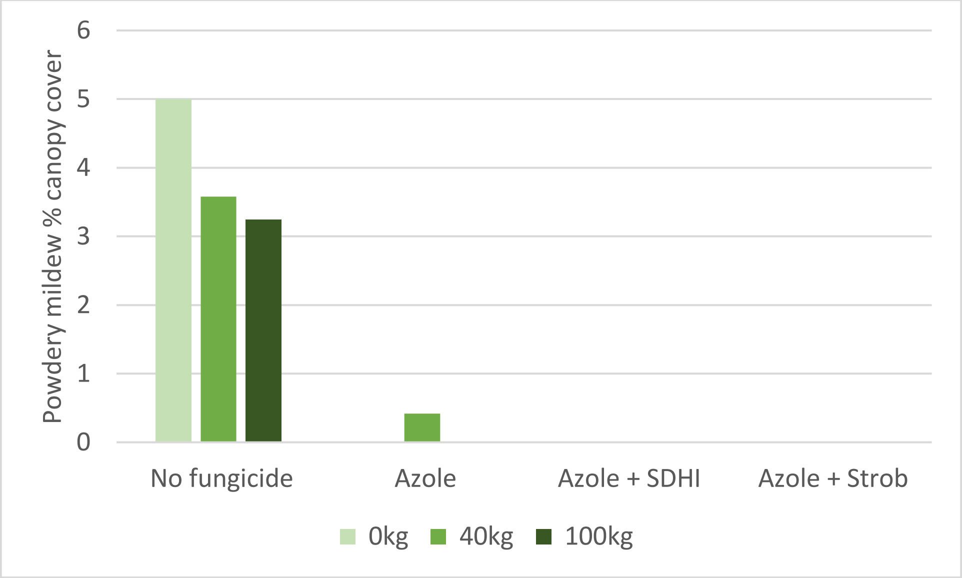

Figure 4.36 Powdery mildew % canopy cover between plants treated with different fungicides, with a range of nitrogen levels, no interaction found, p=0.353).

4.3.2.4 Harvest assessments

There were two hand-harvested biomass harvests carried out during the 2023 field experiment, and one machinery harvest at the very end of the experiment. This section will explore the August biomass harvest first, followed by the December biomass harvest, and finally the machinery harvest.

August biomass harvest:

Leaf weight (dry)

After the leaves were dried, the medium and high nitrogen treatments both resulted in a leaf weight higher than the low nitrogen treatment (p=0.012, **Figure 4.37**).

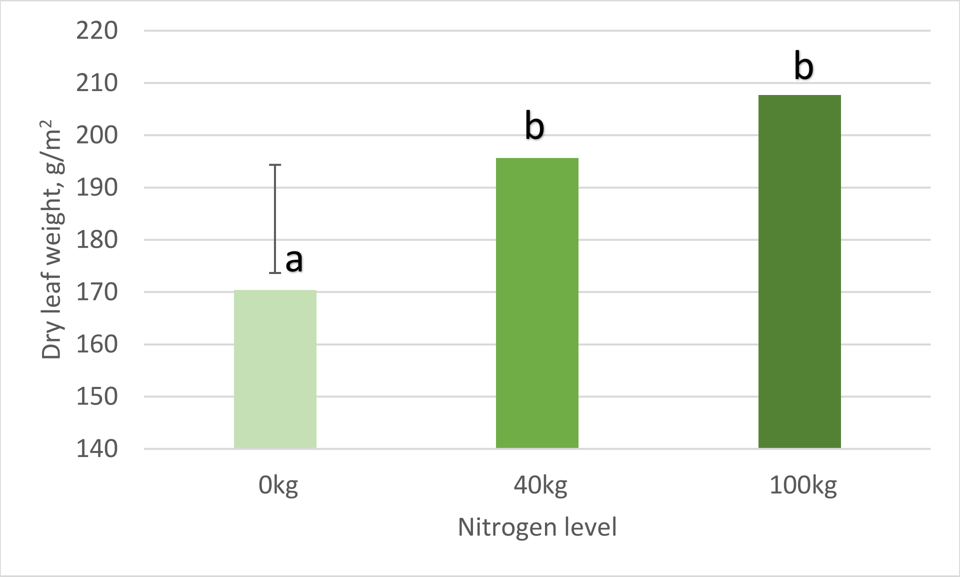

Figure 4.37 Dry weight of leaves from a sample of plots treated with three different doses of nitrogen, p=0.012.

When comparing between fungicide treatments, the azole + SDHI and the azole + strobilurin treatments showed a trend for higher leaf dry weight than the no fungicide control and the azole treatment (p=0.079, **Figure 4.38**).

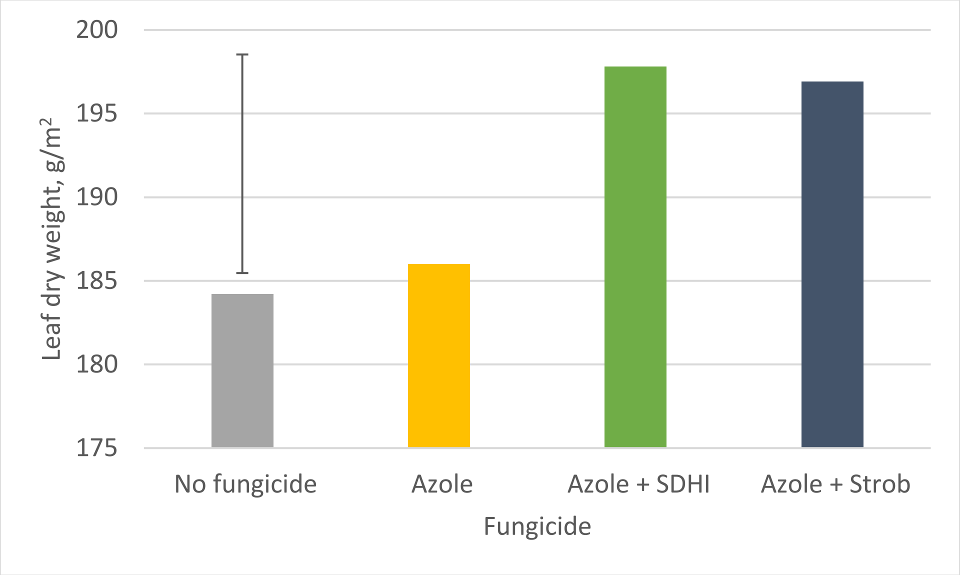

Figure 4.38 Dry weight of leaves from a sample of plots treated with different fungicides, almost statistically significant, p=0.079.

There was no interaction between the effects of nitrogen treatment and fungicide treatment on leaf dry weight (p=0.929, **Figure 4.39**).

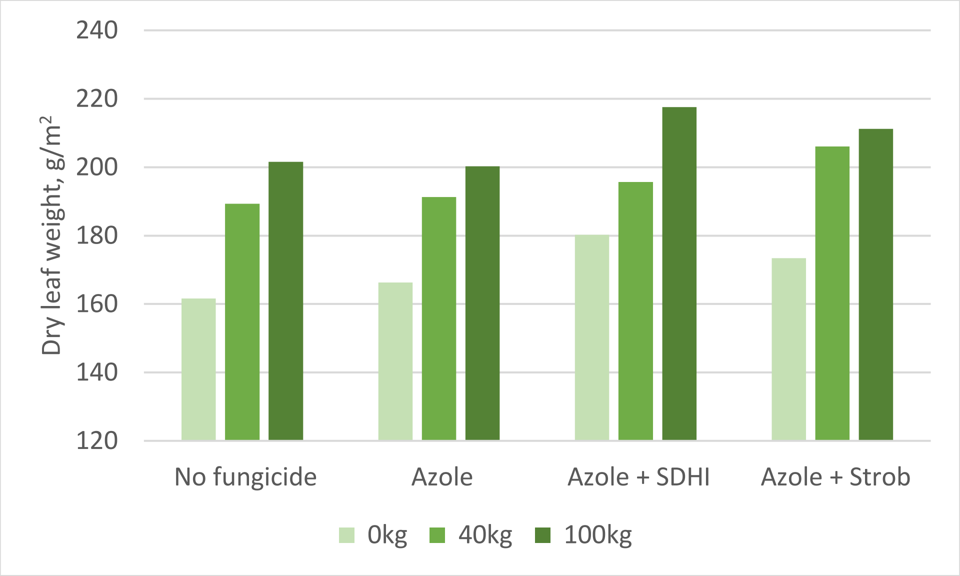

Figure 4.39 Effects of fungicides on leaf dry weight, at each of the three nitrogen levels, no significant interaction found (p=0.929).

Petiole weight (dry)

The high and medium nitrogen levels had significantly higher petiole dry weight than the low nitrogen level (p=0.009, **Figure 4.40**).

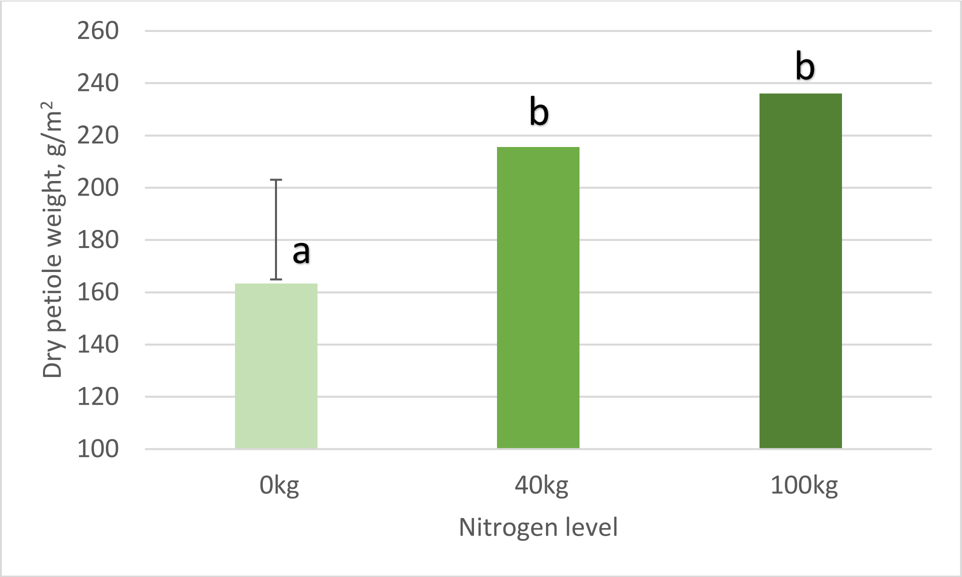

Figure 4.40 Dry weight of petioles from a sample of plots treated with three levels of nitrogen, p=0.009.

There was no significant difference in petiole dry weight between the fungicide treatments (p=0.446).

There was no interaction observed between the effects of nitrogen treatment and fungicide treatment on petiole dry weight (p=0.677, **Figure 4.41**).

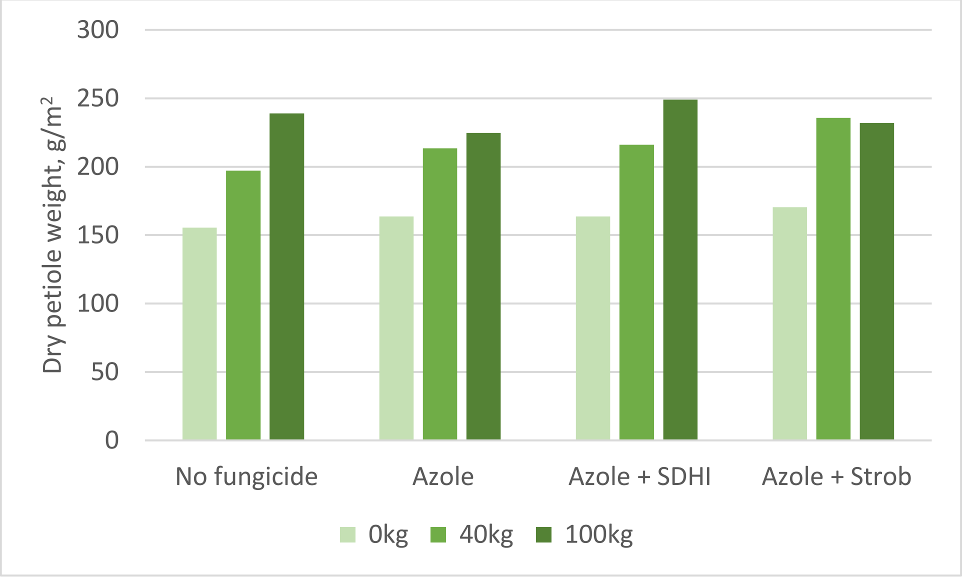

Figure 4.41 Effects of fungicides on petiole dry weight, at each of the three nitrogen levels, no significant interaction found (p=0.677).

Root dry weight

The high nitrogen treatment resulted in a higher root dry weight than the low nitrogen treatment (p=0.027, **Figure 4.42**).

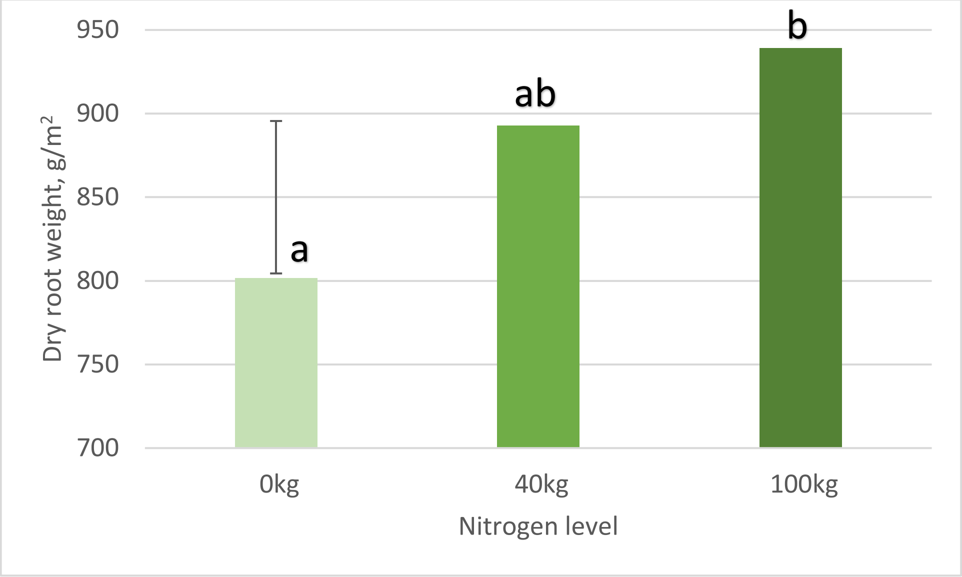

Figure 4.42 Dry weight of roots from a sample of plots treated with three different doses of nitrogen, p=0.027.

There was no significant difference in root dry weight between the fungicide treatments (p=0.302).

There was no interaction between the effects of nitrogen treatment and fungicide treatment on root dry weight (p=0.536, **Figure 4.43**).

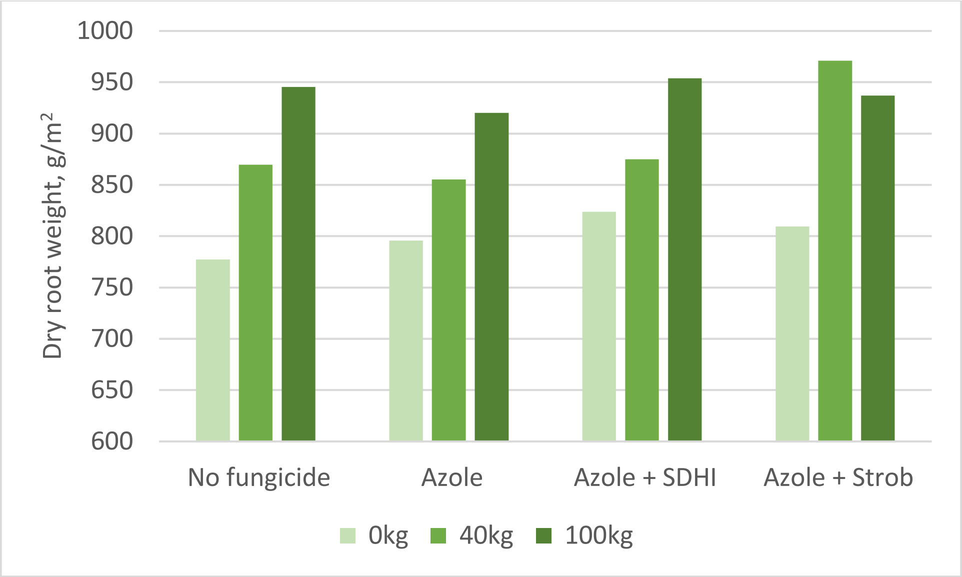

Figure 4.43 Effects of fungicides on root dry weight at each of the three nitrogen levels, no significant interaction found (p=0.536).

Root:top ratio (dry weight)

When comparing the ratio between root weight and top weight, the low nitrogen treatment had a trend for a higher ratio than the high nitrogen treatment (p=0.061, **Figure 4.44**).

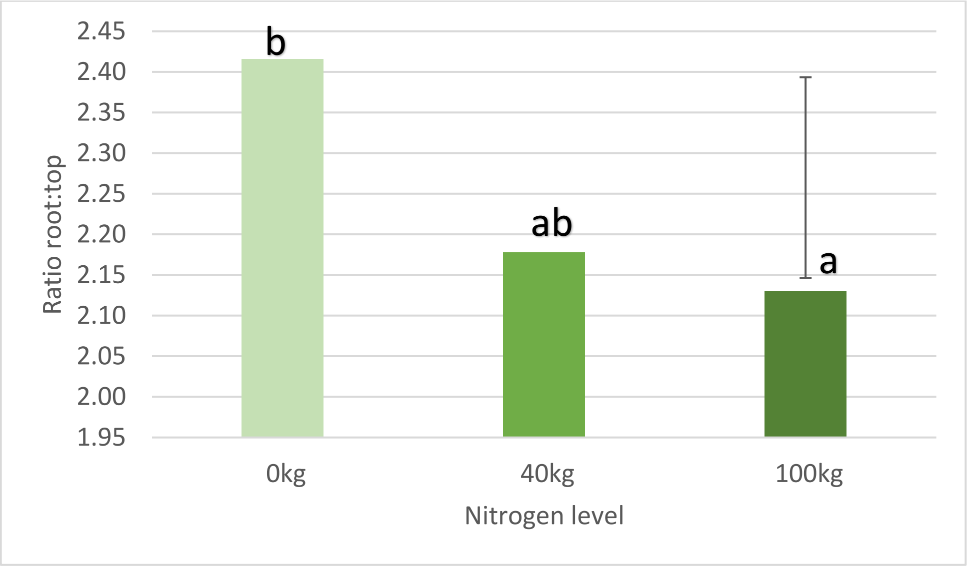

Figure 4.44 Dry root:top ratio of plants from a sample of plots treated with three different doses of nitrogen, p=0.061.

This indicated that for the low nitrogen treatment, the root made up more of the total weight than in the high nitrogen plants. At this stage in the season, the roots were small compared to their size at final harvest, so differences in leaf and petiole weights have more bearing on this ratio at this stage.

There was no significant difference in root:top ratio between the fungicide treatments (p=0.491, **Figure 4.45**).

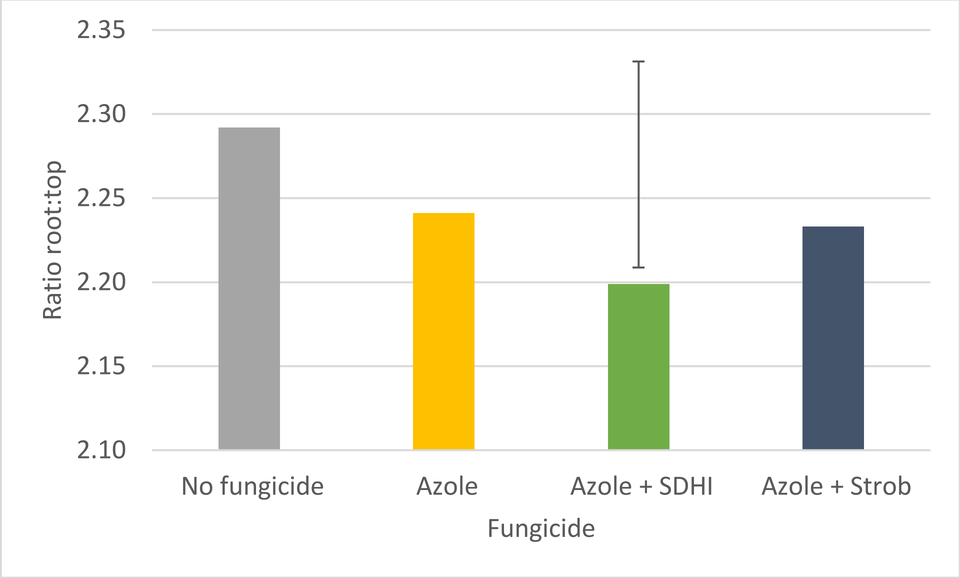

Figure 4.45 Root:top ratio from a sample of plots treated with different fungicides, no significant differences found, p=0.491.

There was no interaction observed between the effects of nitrogen treatment and fungicide treatment on root:top ratio (p=0.915, **Figure 4.46**).

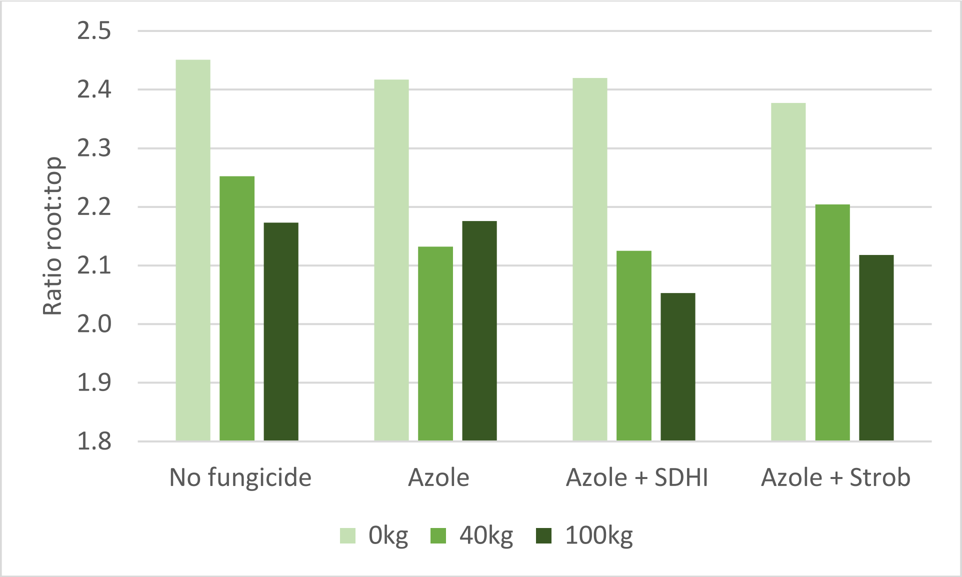

Figure 4.46 Effects of fungicides on root:top ratio at each of the three nitrogen levels, no significant interaction found (p=0.915).

December biomass harvest

Leaf weight (dry)

There was no significant difference in dry leaf weight found between the nitrogen levels (p=0.322), however the results matched the ranking from the August biomass harvest, when there was a significant difference (high N > medium N > low N) (**Figure 4.47**).

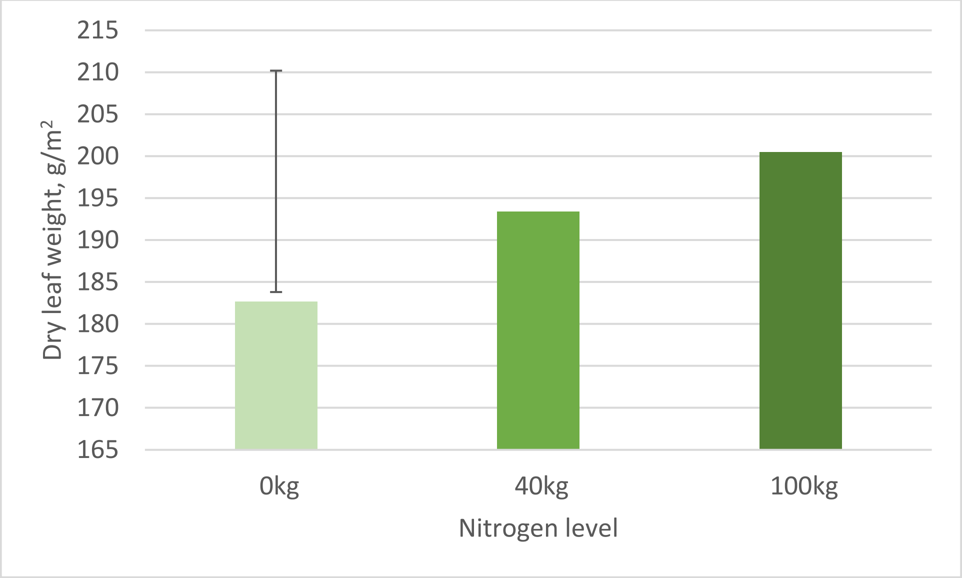

Figure 4.47 Leaf dry weights of plants treated with three different doses of nitrogen, no significant difference found, p=0.322.

There was no significant difference in leaf dry weight between the different fungicide treatments (p=0.383).

There was no interaction observed between the effects of nitrogen treatment and fungicide treatment on leaf dry weight (p=0.930, **Figure 4.48**).

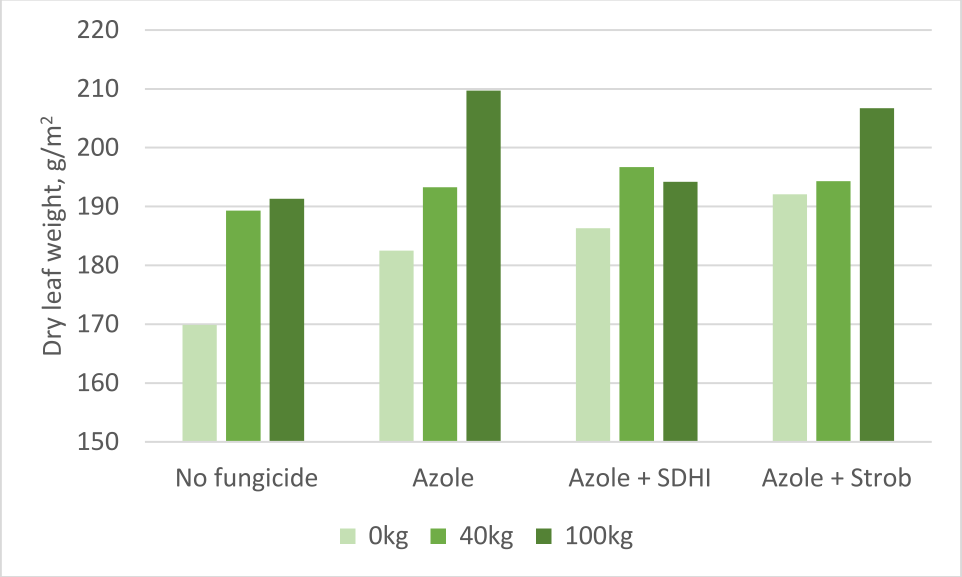

Figure 4.48 Effects of fungicides on leaf dry weight at each of the three nitrogen levels, no significant interaction found (p=0.930).

Petiole dry weight

There was no difference in petiole dry weight found between the nitrogen levels (p=0.198), however, the results matched the ranking from the August biomass harvest, when there was a significant difference (high N > medium N > low N) (**Figure 4.49**).

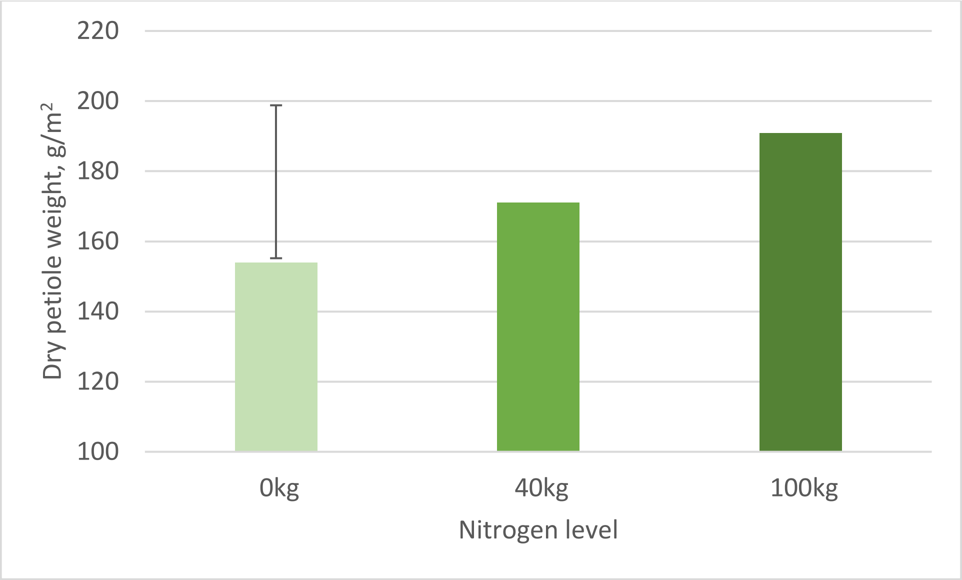

Figure 4.49 Petiole dry weights of plants from a sample of plots treated with three different doses of nitrogen, no significant difference found, p=0.198.

There was no difference in dry petiole weight between the fungicide treatments (p=0.788).

There was no interaction observed between the effects of nitrogen treatment and fungicide treatment on petiole dry weight (p=0.654, **Figure 4.50**).

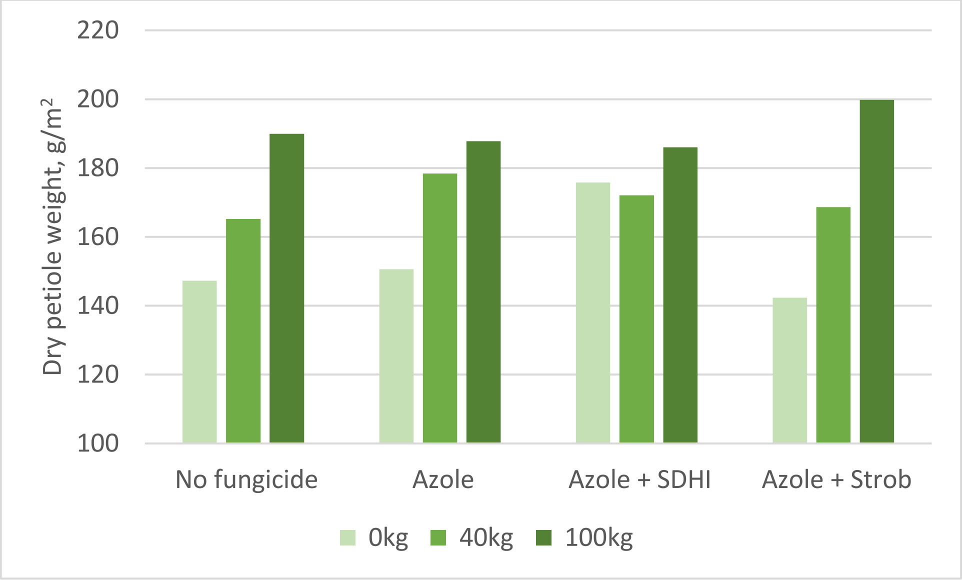

Figure 4.50 Effects of fungicides on petiole dry weight at each of the three nitrogen levels, no significant interaction found (p=0.654).

Root dry weight

There was no difference in dry root weight between the nitrogen levels (p=0.147), however the results matched the ranking from the August biomass harvest, when there was a significant difference (high N > medium N > low N) (**Figure 4.51**).

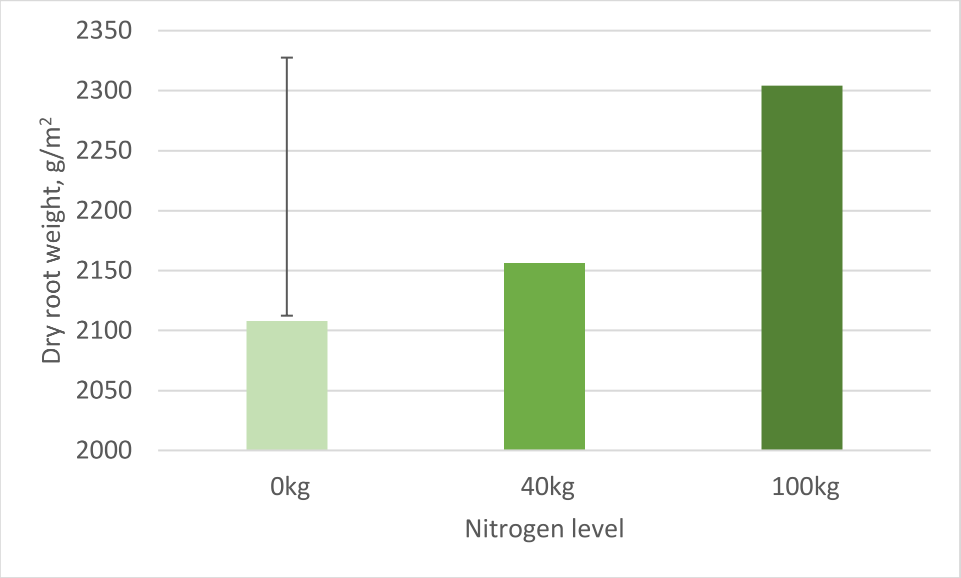

Figure 4.51 Root dry weights of plants treated with three different doses of nitrogen, no significant difference found, p=0.147.

There was a trend for a difference in root dry weight between the fungicide treatments (p=0.101, **Figure 4.52**), with the azole + SDHI treatment resulting in the highest weight and the azole + strobilurin treatment the lowest weight.

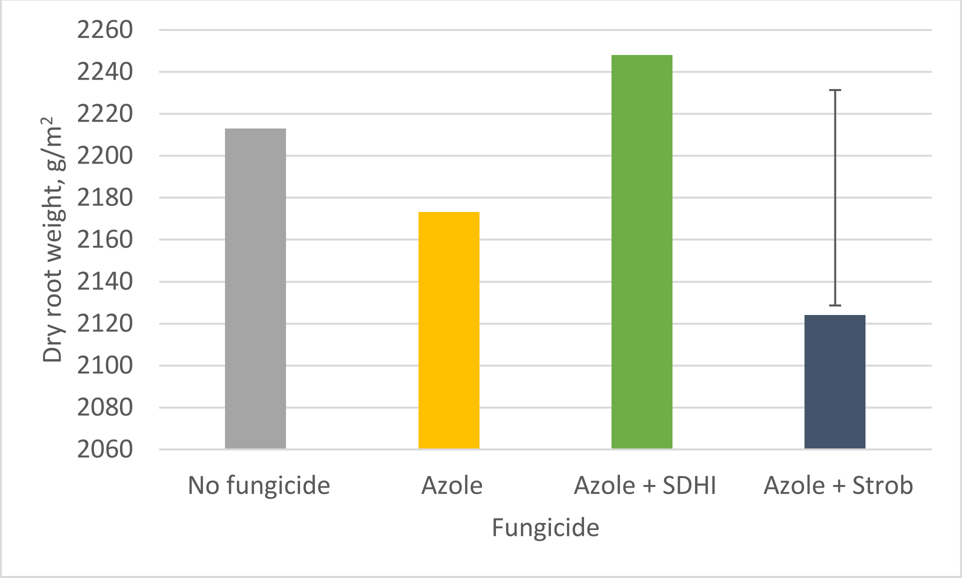

Figure 4.52 Root dry weights of plants from a sample of plots treated with different fungicides, no significant difference found, p=0.101.

There was no interaction between the effects of nitrogen treatment and fungicide treatment on root dry weight (p=0.290, **Figure 4.53**).

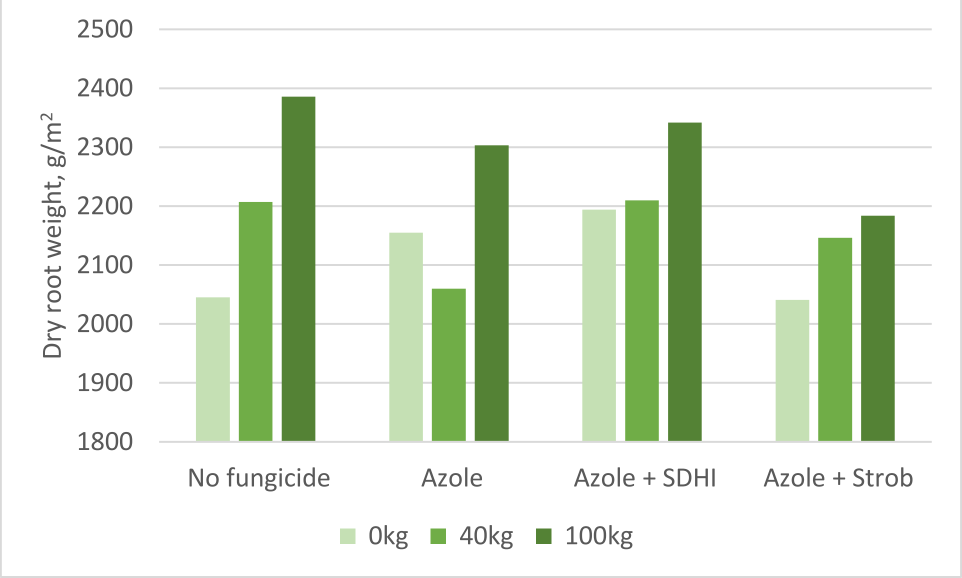

Figure 4.53 Effects of fungicides on root dry weight at each of the three nitrogen levels, no significant interaction found (p=0.290).

Dry weight root:top ratio

When comparing the ratio between root weight and top weight, there was no difference between nitrogen levels, however the ranking of the treatments was the same as in the

August biomass harvest, when there were significant differences present (low N > medium N > high N) (p=0.366, **Figure 4.54**).

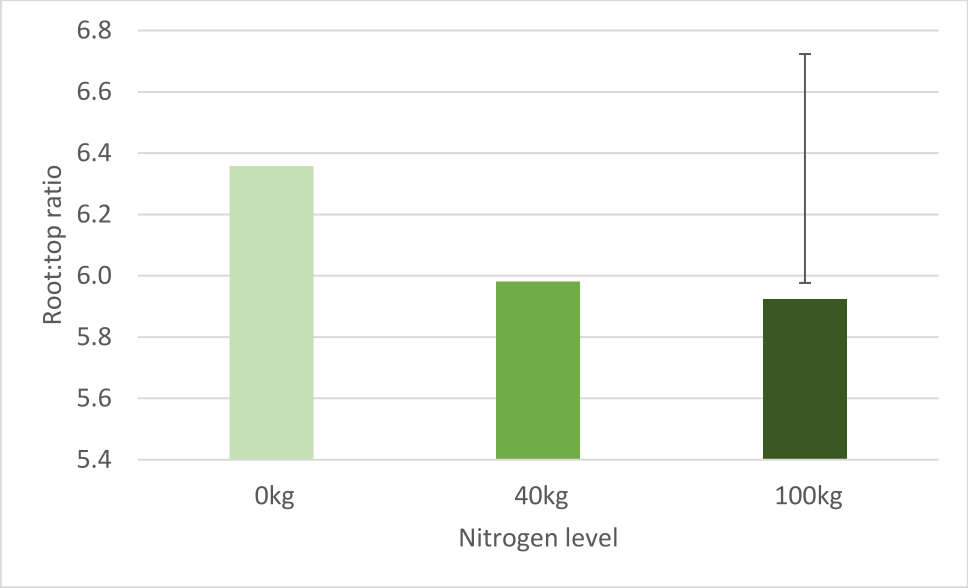

Figure 4.54 Dry root:top ratio of plants from a sample of plots treated with three different doses of nitrogen, no significant differences found, p=0.366.

There was no significant difference in root:top ratio between the fungicide treatments (p=0.237, **Figure 4.55**).

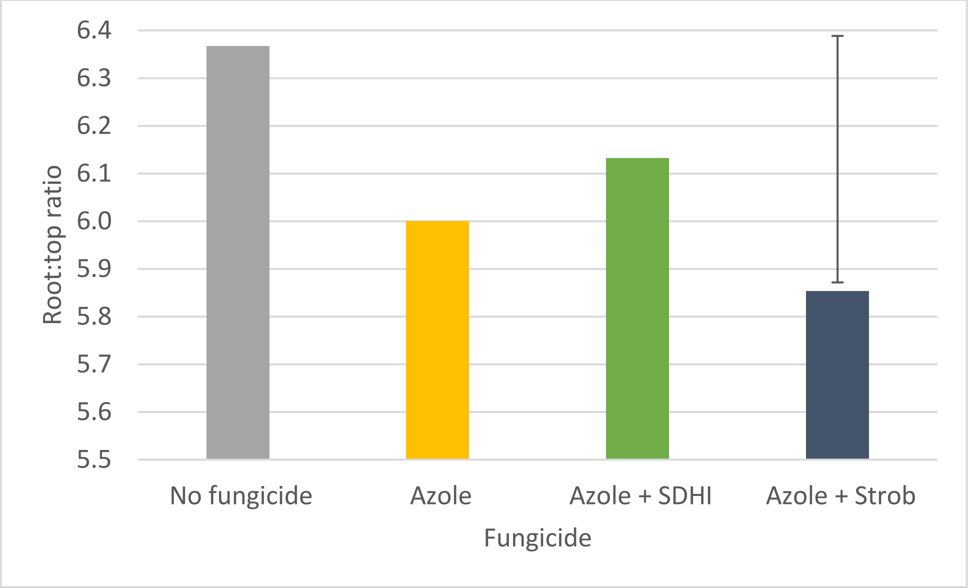

Figure 4.55 Root:top ratio (dry weight) from a sample of plots treated with different fungicides, no significant differences found, p=0.237.

There was no interaction observed between the effects of nitrogen treatment and fungicide treatment on root:top ratio (p=0.486, **Figure 4.56**).

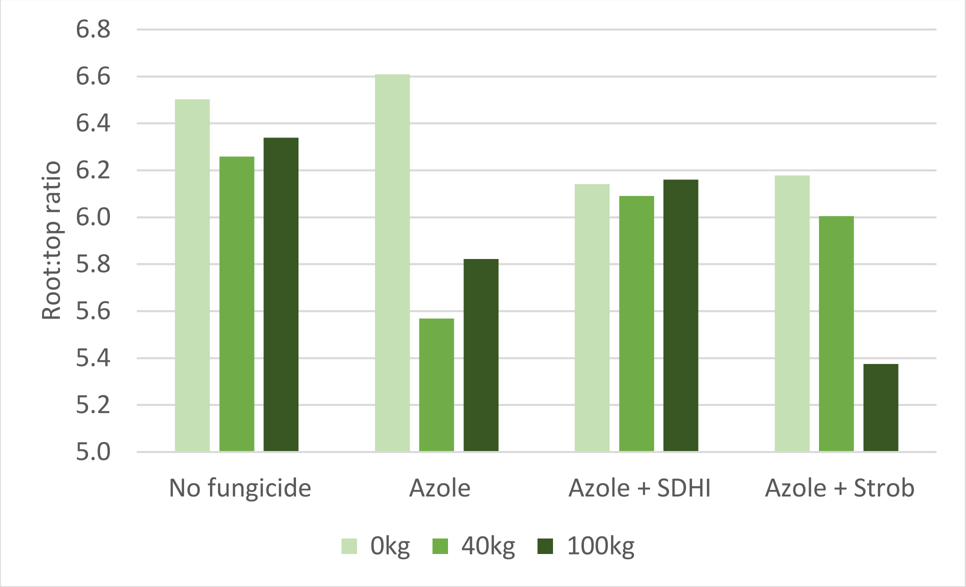

Figure 4.56 Effects of fungicides on root:top ratio at each of the three nitrogen levels, no significant interaction found (p=0.486).

Final machine harvest yield

The following data are from the machinery harvest which took place after the final biomass harvest.

Sugar yield

The machinery harvest data showed that the sugar yield was higher in the high nitrogen plots than in the low nitrogen plots, but the medium nitrogen did not differ statistically from either (p=0.032). The sugar yield was higher with the azole + SDHI treatment and with the azole treatment than for the no fungicide control (p=0.005). There was no interaction between the effects of N level and fungicide treatment (p=0.787, **Figure 4.57**).

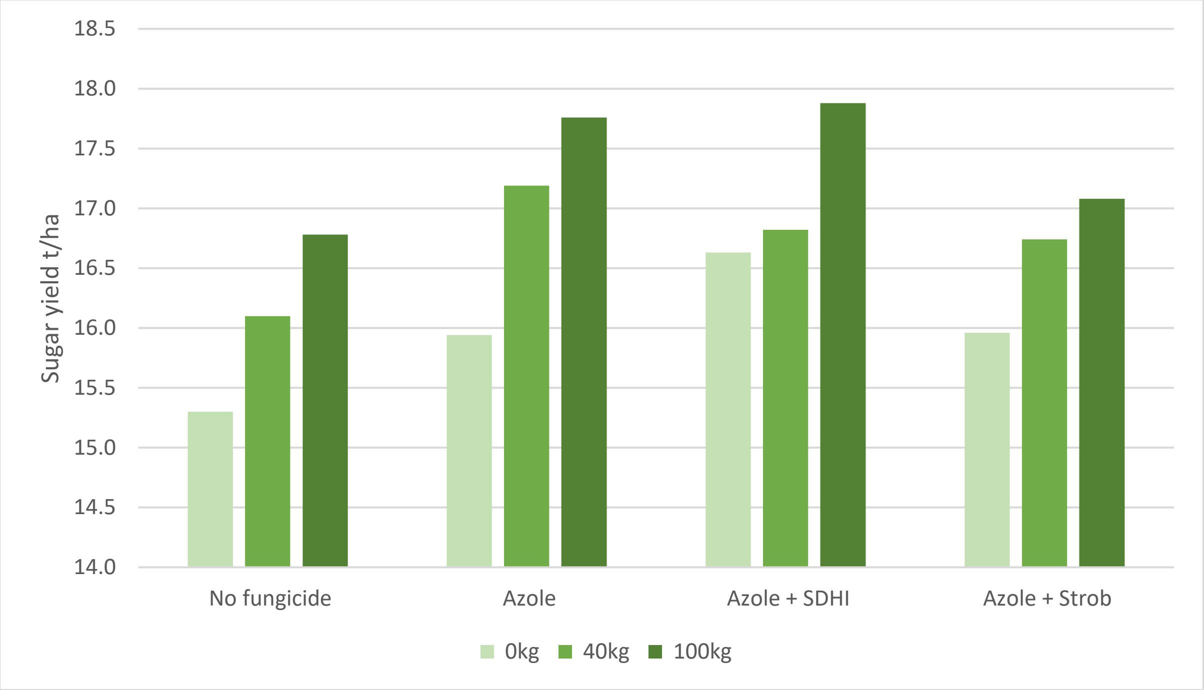

Figure 4.57 Sugar yield results from the machinery harvest of the field experiment 2023, comparing between nitrogen application levels and fungicide treatments. No interaction observed (p=0.787).

Impurities

Impurities in the root can affect the extractability of sugar and subsequently reduce final sugar yields.

Amino nitrogen content was higher in the high nitrogen plots than in the medium and low nitrogen plots (p=0.009, **Figure 4.58**).

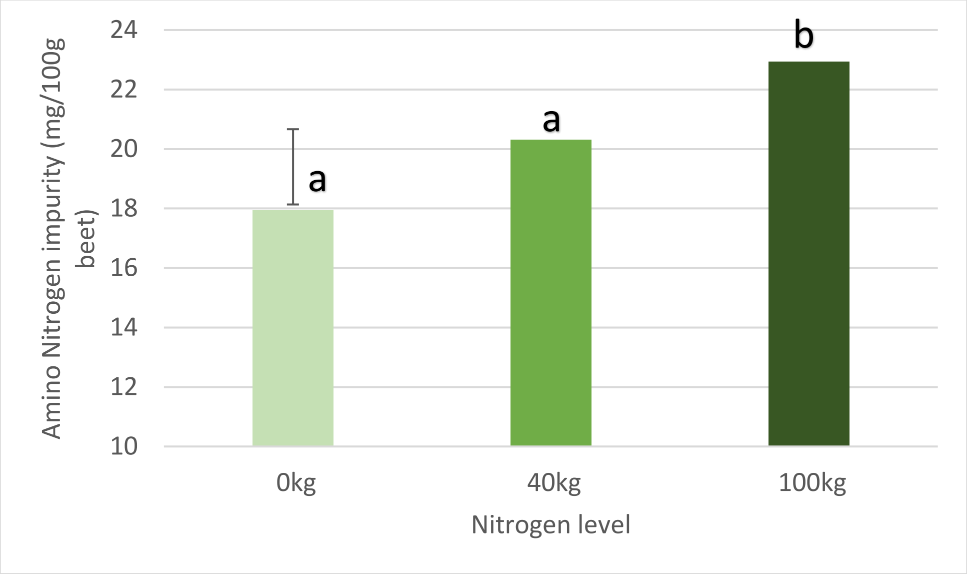

Figure 4.58 Amino nitrogen impurity content in plants treated with three different nitrogen doses, p=0.009.

There was no significant difference in amino nitrogen content between fungicide treatments (p=0.628). There was no interaction observed between the effects of nitrogen treatment and fungicide treatment on amino nitrogen impurity content (p=0.564, **Figure 4.59**).

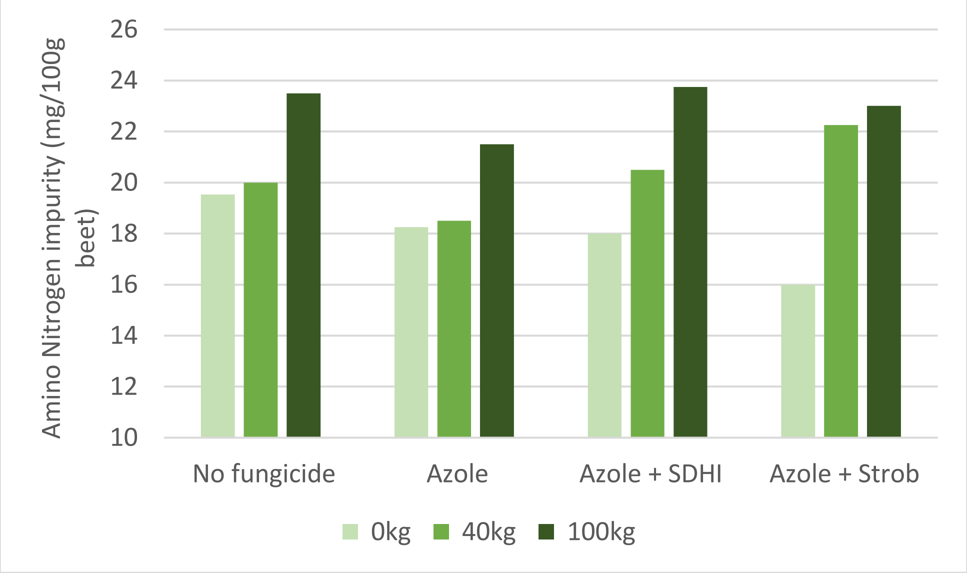

Figure 4.59 Effects of fungicides on amino nitrogen impurity content at each of the three nitrogen levels, no significant interaction found (p=0.564).

There was no significant difference in potassium impurity content between nitrogen levels (p=0.272, **Figure 4.60**).

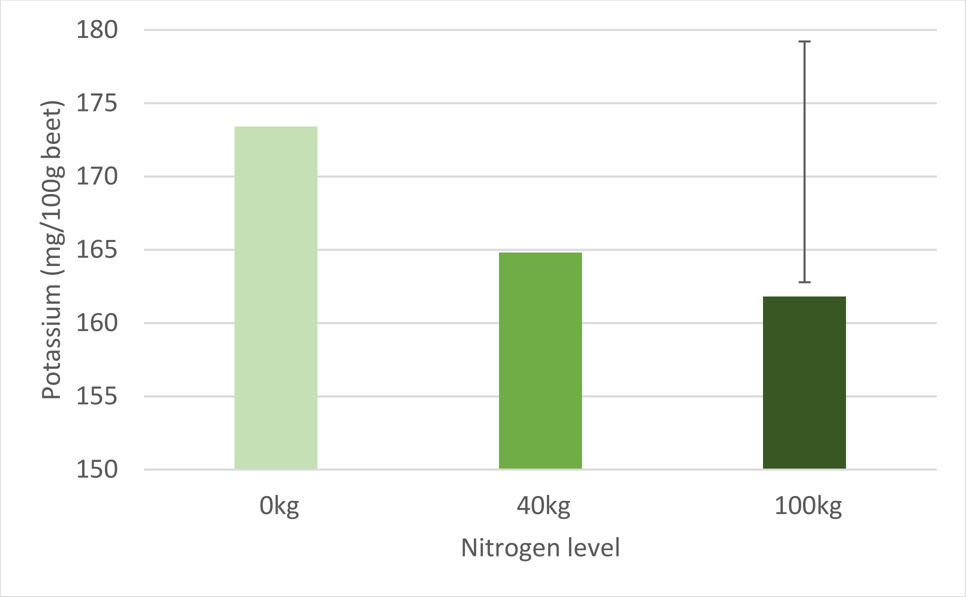

Figure 4.60 Potassium impurity content in plants treated with different nitrogen levels, no significant differences found, p=0.272.

There were no differences in potassium impurity content between fungicide treatments (p=0.772). No interaction was observed between the effects of nitrogen treatment and fungicide treatment on potassium impurity content (p=0.885, **Figure 4.61**).

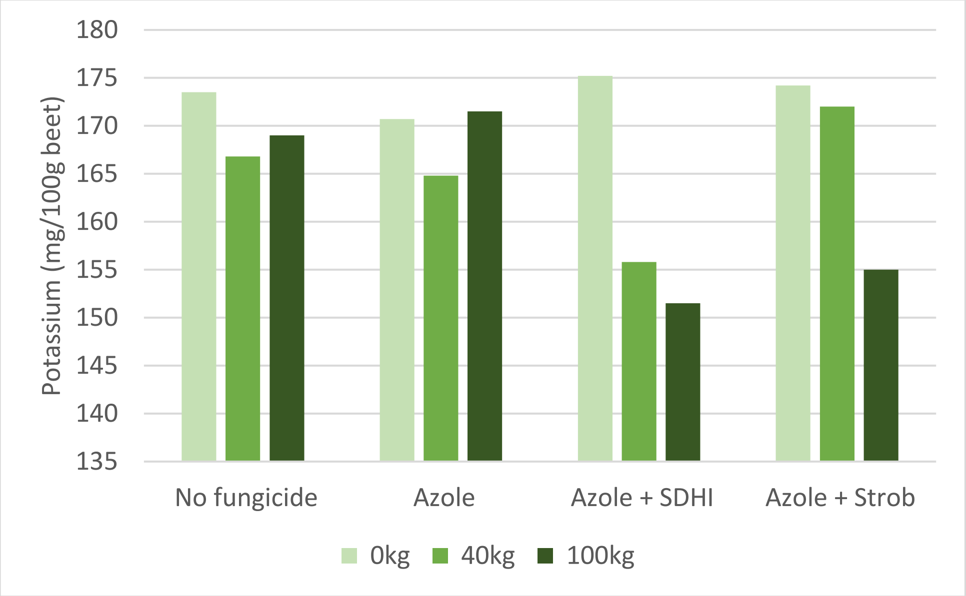

Figure 4.61 Effects of fungicides on potassium impurity content at each of the three nitrogen levels, no significant interaction found (p=0.885).

Sodium content in the roots was lower in the medium nitrogen treatment than in the low and high nitrogen treatments (p=0.026, **Figure 4.62**).

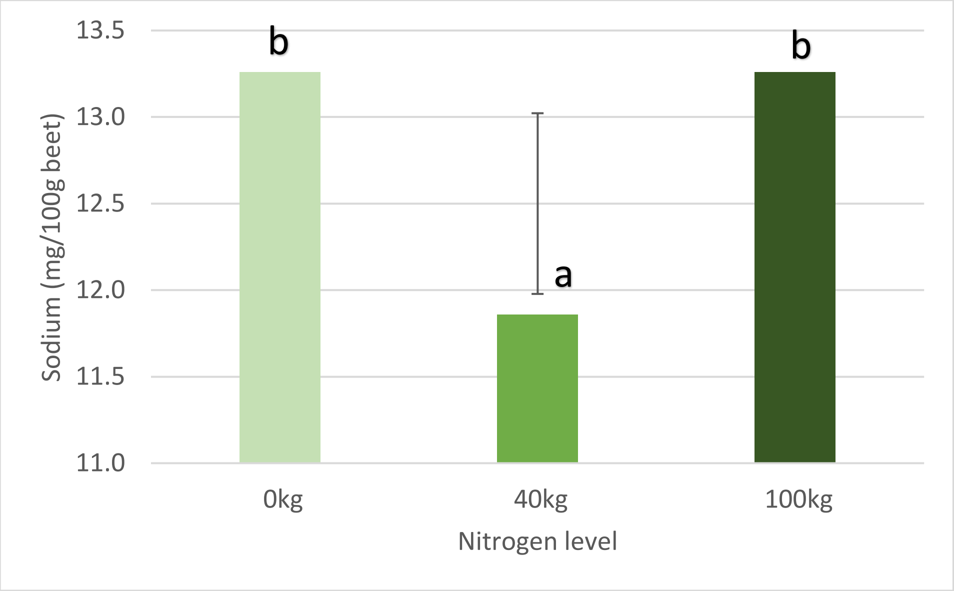

Figure 4.62 Sodium impurity content in plants treated with different nitrogen levels, p=0.026.

There were no significant differences in sodium content in the roots between fungicide treatments (p=0.290). There was an interaction observed between the effects of nitrogen treatment and fungicide treatment on sodium impurity content (p=0.046, **Figure 4.63**).

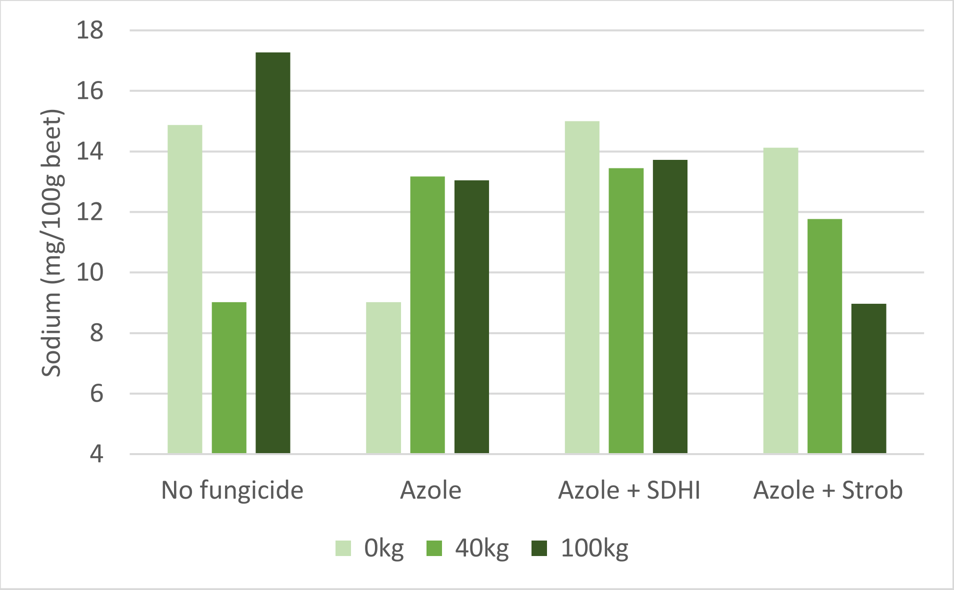

Figure 4.63 Effects of fungicides on Sodium impurity content at each of the three nitrogen levels, p=0.046.

4.3.3 Glasshouse experiment

4.3.3.1 Leaf chlorophyll content (SPAD)

Using a repeated measures analysis of the 2024 glasshouse SPAD data, there was an interaction between the effects of fungicide and nitrogen level on leaf SPAD values (p=0.042, **Figure 4.64**). The treatment with the lowest leaf SPAD was the low nitrogen no-fungicide control, and all of the high nitrogen treatments, regardless of fungicide, had significantly higher leaf SPAD than this treatment. Under the low nitrogen treatment, the azole + strobilurin treatment was not significantly different from the no fungicide treatment, however the azole + SDHI treatment had significantly higher leaf SPAD, and was not significantly different from the high nitrogen treatment for this fungicide treatment.

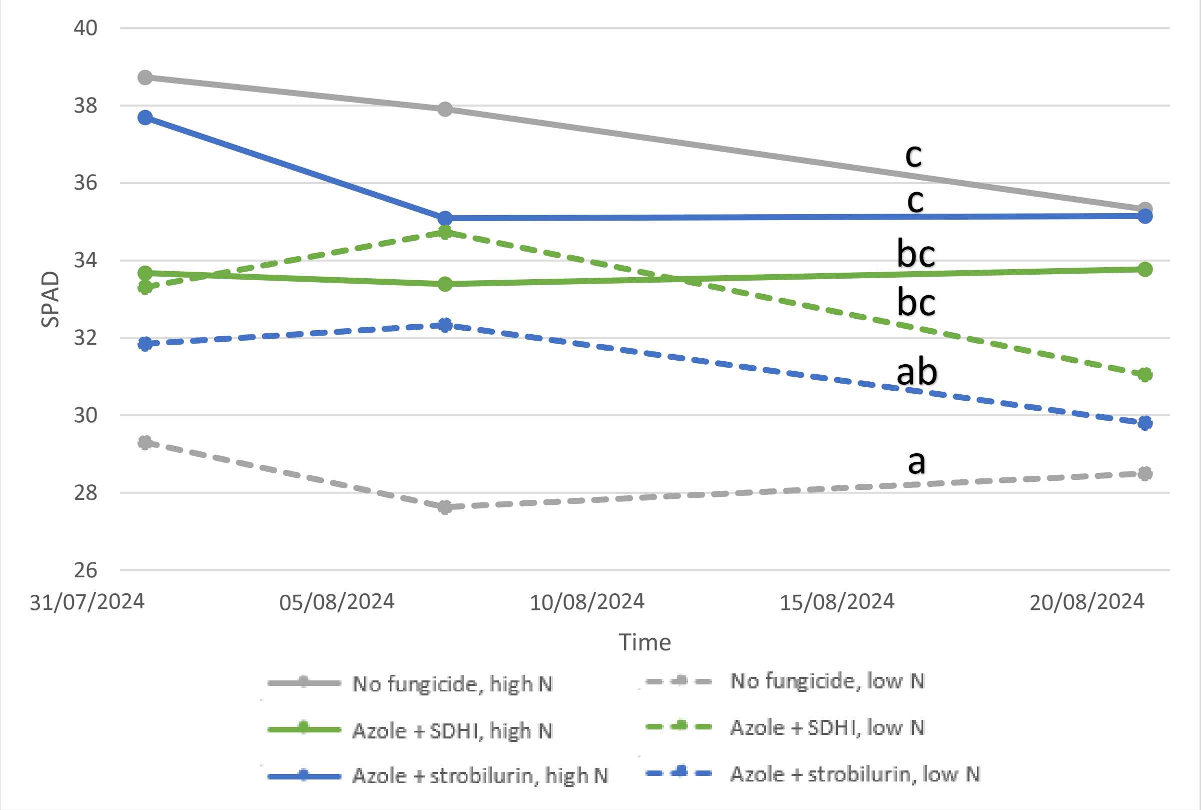

Figure 4.64 Leaf SPAD for three different fungicide treatments under two nitrogen levels, p=0.042 using repeated measures analysis. Multiple comparisons calculated using Fisher's protected least significant difference test shown using letters a-c.

When all dates were included in the repeated measures analysis (as opposed to the previous result where only dates after fungicide application were included), there was a significant difference in leaf SPAD between the two nitrogen levels (p=0.001, **Figure 4.65**).

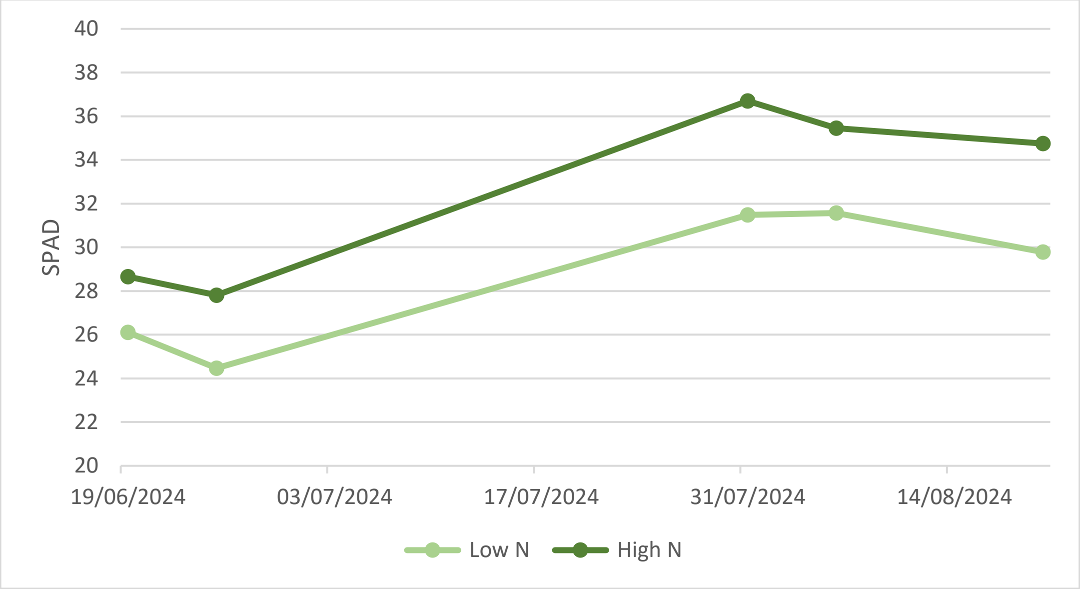

Figure 4.65 Leaf SPAD for two nitrogen levels across time, p=0.001.

4.3.3.2 Leaf photosynthetic activity (LI-COR)

Leaf CO_2 assimilation rate

Using a repeated measures analysis of data from after fungicide treatments only, there was no significant interaction between the effects of fungicide and nitrogen level on leaf CO_2 assimilation rate (p=0.540, **Figure 4.66**). When all dates were included (not only dates after fungicide application), high nitrogen plants had higher CO_2 assimilation rate than low nitrogen plants (P<0.001). Using only data after fungicide application, there were no significant differences between fungicide treatments (p=0.517).

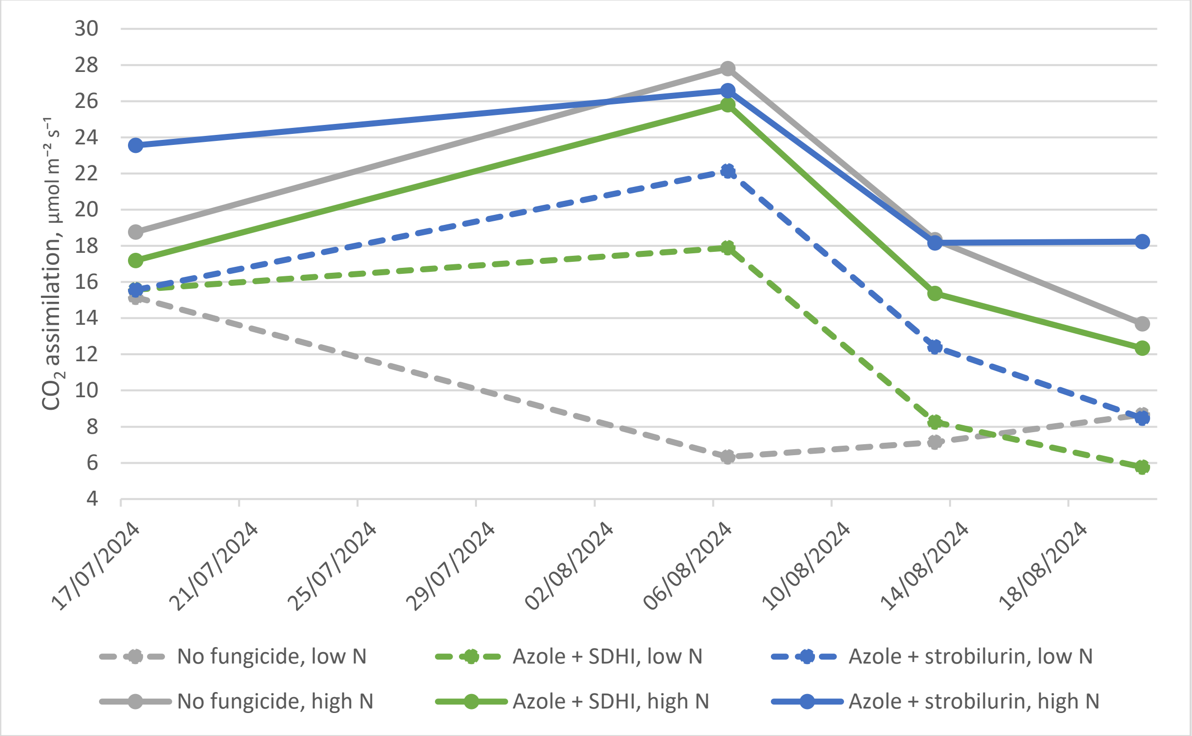

Figure 4.66 Leaf CO_2 assimilation rates for plants treated with different fungicides and either high or low nitrogen fertiliser. No interaction observed (p=0.517).

Leaf stomatal conductance

Using a repeated measures analysis of data from after fungicide treatments only, there was no significant interaction between the effects of fungicide and nitrogen level on stomatal conductance (p=0.123, **Figure 4.67**). When all dates were included (not only dates after fungicide application), high nitrogen plants had significantly higher stomatal conductance than low nitrogen plants (P<0.001). Using only data after fungicide application, there were no significant differences between fungicide treatments (p=0.123).

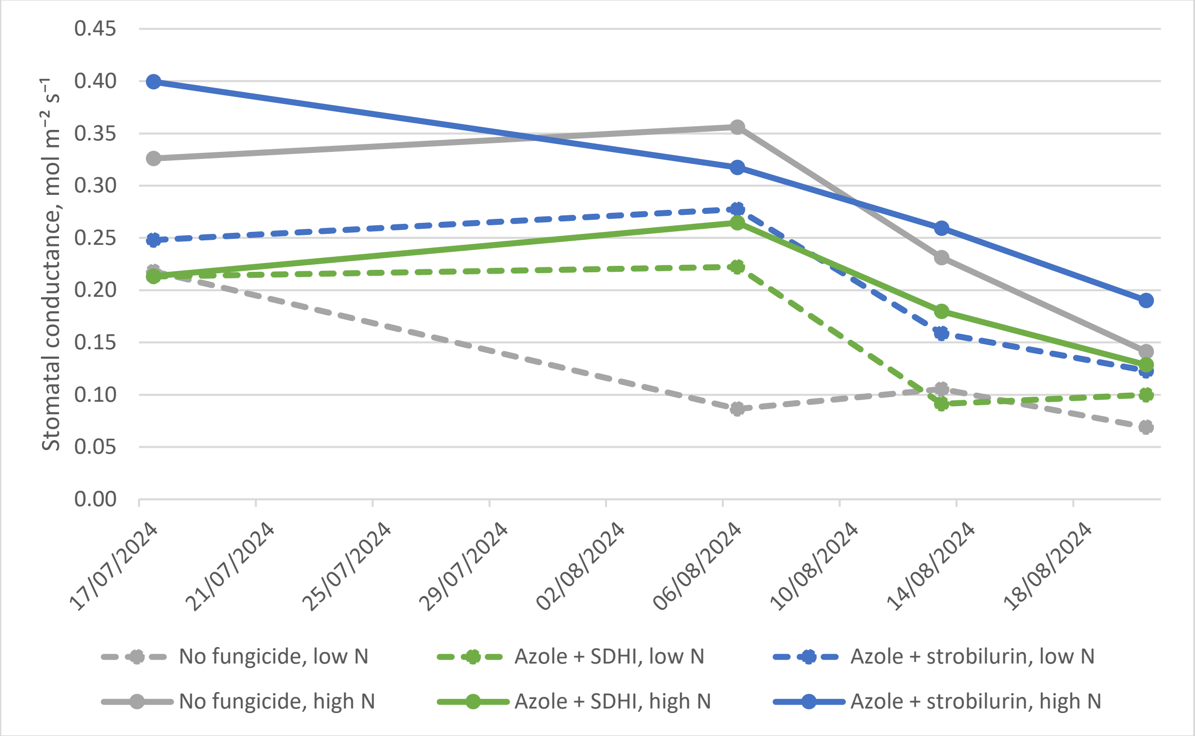

Figure 4.67 Leaf stomatal conductance for plants treated with different fungicides and either high or low nitrogen fertiliser. No interaction observed (p=0.123).

Chlorophyll fluorescence

Using a repeated measures analysis of data from after fungicide treatments only, there was no significant interaction between the effects of fungicide and nitrogen level on leaf chlorophyll fluorescence quantum yield (p=0.346, **Figure 4.68**). When all dates were included (not only dates after fungicide application), high nitrogen plants had significantly higher chlorophyll fluorescence than low nitrogen plants (p<0.001). Using only data after fungicide application, there were no significant differences between fungicide treatments (p=0.385).

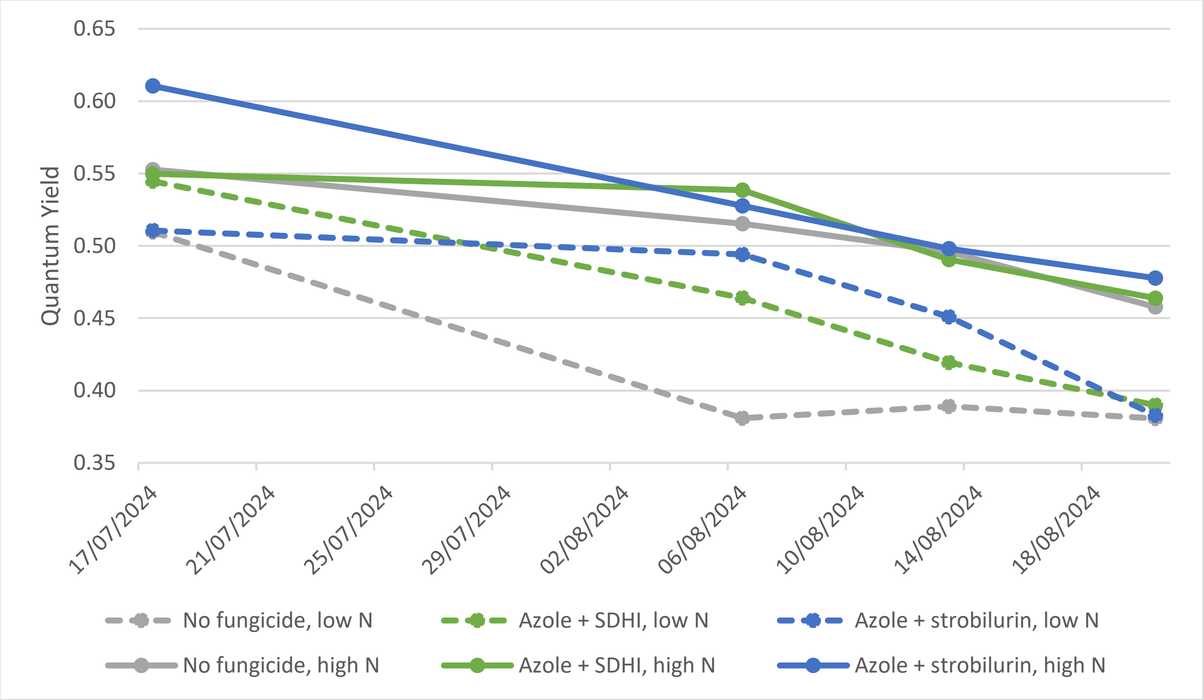

Figure 4.68 Chlorophyll fluorescence quantum yield of plants treated with different fungicides and either high or low nitrogen. No interaction observed (p=0.385).

4.3.3.3 Harvest assessments

There was no significant interaction between the effects of fungicide and nitrogen levels on root dry weight (p=0.474, **Figure 4.69**). The roots of low nitrogen plants weighed significantly less than the roots of high nitrogen plants (p<0.001).

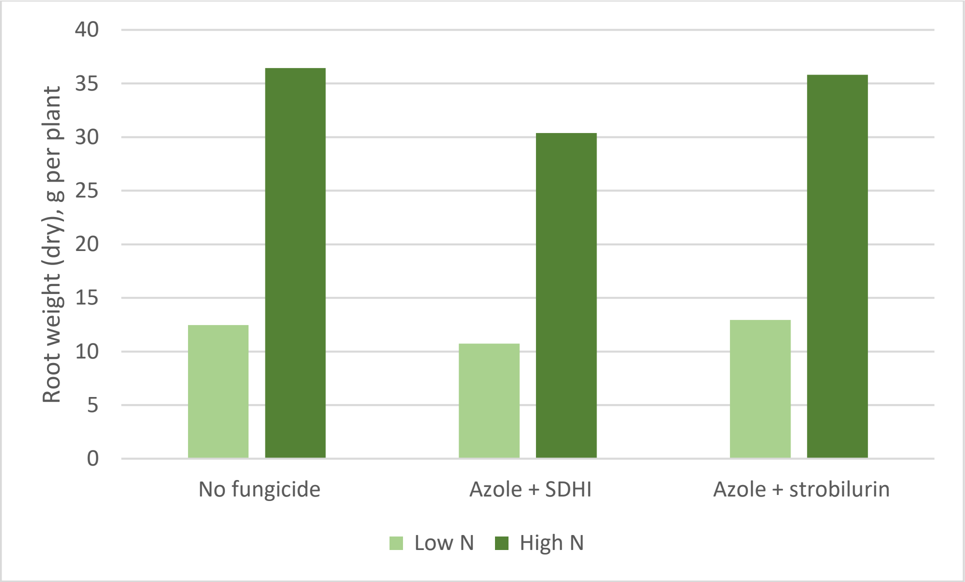

Figure 4.69 Root dry weights of plants treated with different fungicides and two different levels of nitrogen fertiliser, no interaction present (p=0.474).

The roots of plants from azole + SDHI treated plants weighed less than those which received no fungicide treatment, and those which were treated with azole + strobilurin, however this was not significant (p=0.076, **Figure 4.70**).

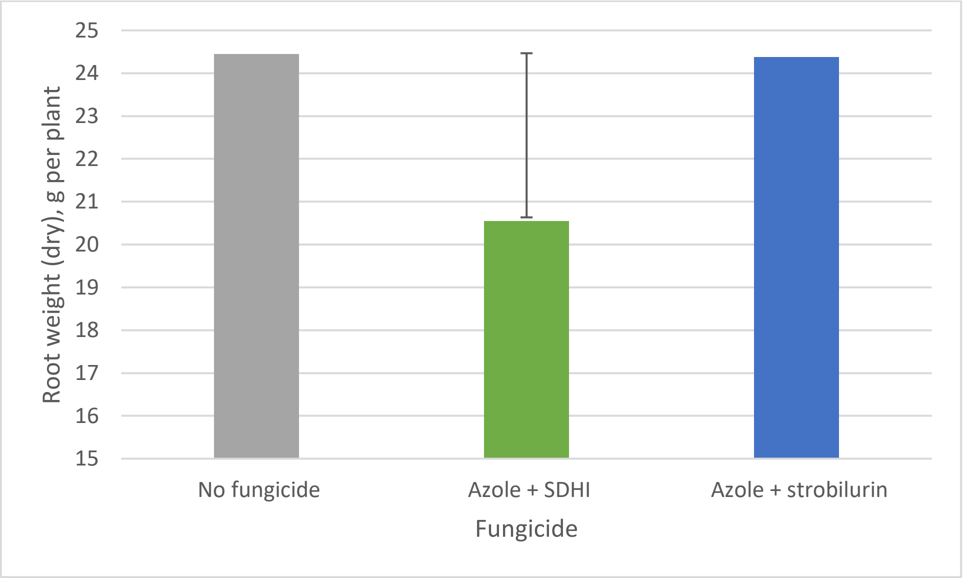

Figure 4.70 Root dry weights of plants treated with different fungicides, not statistically significant (p=0.076).

There was no significant interaction between the effects of fungicide and nitrogen levels on leaf weight (p=0.459, **Figure 4.71**). The leaves of low nitrogen plants weighed significantly less than the leaves of high nitrogen plants (p<0.001).

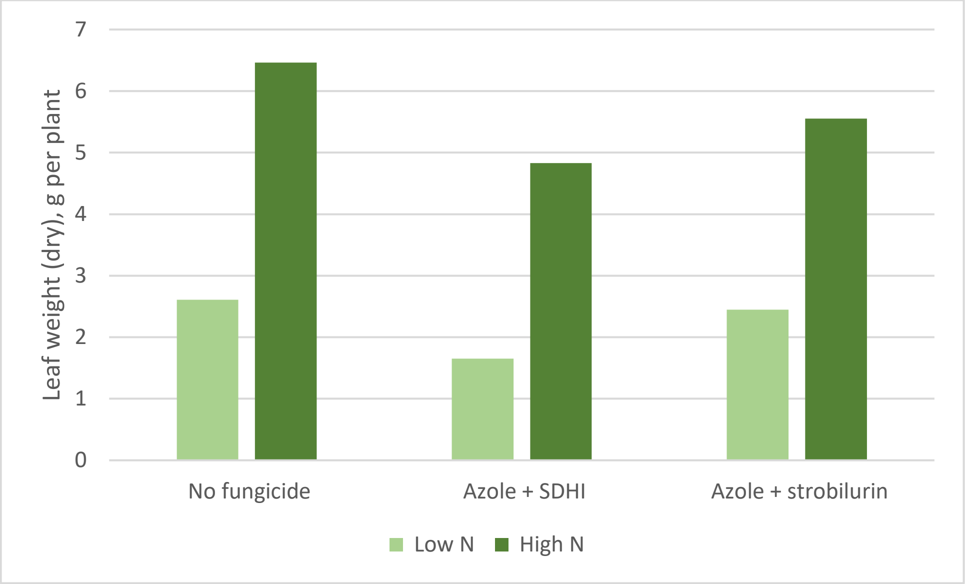

Figure 4.71 Leaf dry weight of plants treated with different fungicides and two different levels of nitrogen fertiliser, no interaction present (p=-0.459).

The leaves of plants treated with azole + SDHI weighed less than the leaves of plants treated with azole + strobilurin and the untreated plants (p=0.004, **Figure 4.72**).

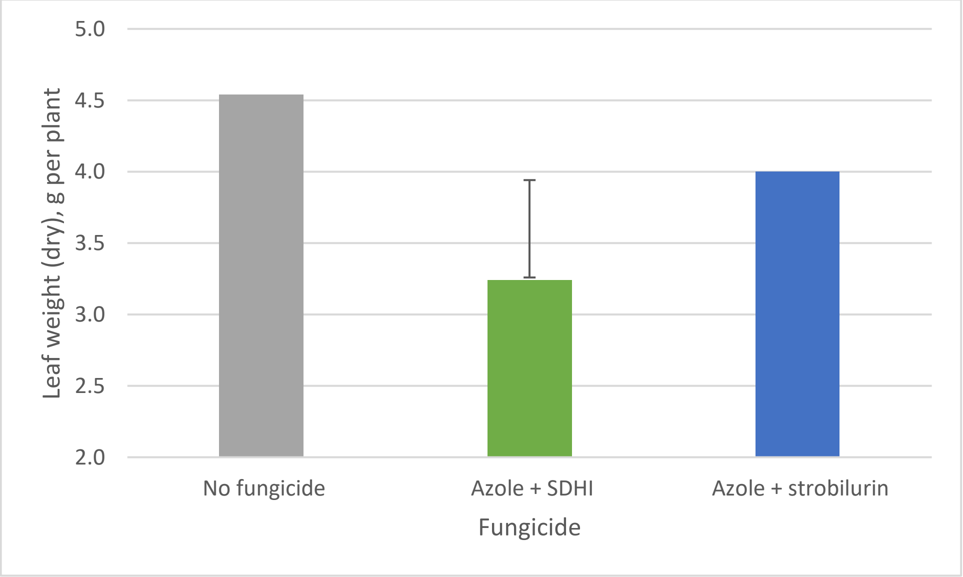

Figure 4.72 Leaf dry weights of plants treated with different fungicides, p=0.004.

There was an interaction between the fungicide treatment and nitrogen level on petiole weight (p=0.059, **Figure 4.73**). Under low nitrogen treatment, all three fungicide treatments resulted in similar petiole weight, but under high nitrogen treatment, the azole + strobilurin treatment resulted in a lower petiole weight than the other two treatments.

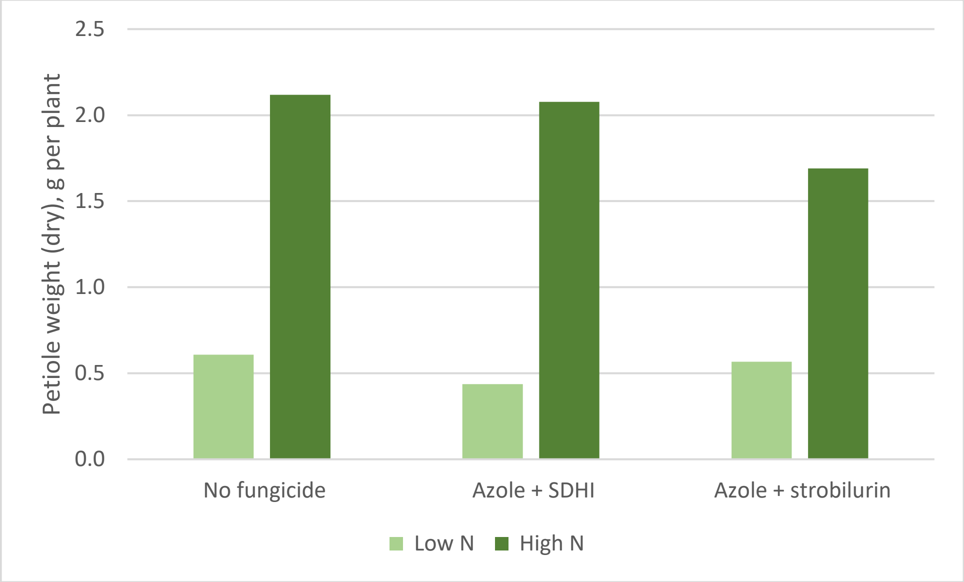

Figure 4.73 Petiole dry weights of plants treated with different fungicides and two different nitrogen fertiliser levels, interaction present (p=0.059).

The petioles of plants grown with low nitrogen weighed significantly less than the petioles of plants grown with high nitrogen (p<0.001, data not shown).

4.4 Discussion

4.4.1 Polytunnel experiment

The experiments covered in this chapter were joined by their aim of identifying the effects of fungicides on sugar beet’s ability to cope with insufficient nitrogen, however the outcomes of each experiment varied. There were limitations with the first experiment, the polytunnel trial in 2022; as the soil in the polytunnel boxes was freshly turned soil and had high levels of available nitrogen (151 kg/ha available at sampling date in March 2022).

The experiment took place regardless of this potential limitation, as it’s not unusual for N availability to decrease e.g. due to immobilisation in the soil bacteria, and there was a possibility that the N availability may decrease enough through the season to observe responses to N fertiliser. However, the results from this experiment indicated that the soil nitrogen had likely remained high, and reduced any differences which may have appeared in more suitable experimental conditions.

There were some trends for differences when looking at the leaf spectral reflectance data. Blue green index (BGI) was higher in the SDHI and azole + SDHI treatments than the no fungicide control, suggesting that these treatments had a positive effect on chlorophyll content. The higher carotenoid reflectance index (CRI) in the control and the strobilurin treated plants than the azole + SDHI suggested that there was a stress response occurring in these treatments, as the ratio of carotenoid content compared with chlorophyll was indicative of nutrient deficiency. For CRI, the azole + SDHI treatment was lower than the other treatments, suggesting that this treatment was associated with healthier plants. Normalised difference water index (NDWI) was higher in the strobilurin treatment and the no fungicide control than the SDHI, suggesting that these plants had more efficient water use, however this can sometimes be indicative of leaves which have prioritised expansion rather than leaf thickness and water retention. NPCI being higher in the SDHI and the azole + SDHI suggests a favourable chlorophyll to carotenoid ratio, which fits with the results which were seen for CRI.

The lower sugar % for this experiment in the high N plants suggested that the dose was high enough to produce unfavourable conditions for the sugar beet, and that the high N had affected sugar content. There was a trend for the high nitrogen plants to have larger leaf area and leaf weight, however, with this lower sugar % it’s possible that the high nitrogen plants had unfavourable partitioning during growth. High N in plants can skew the biomass production towards canopy growth rather than sugar accumulation and storage. An additional contributing factor that may have affected these results is that high nitrogen can affect the extractability of sugar due to added impurity, and so the final sugar % is affected.

The physiological responses suggested that some fungicides, particularly the azole + SDHI and the SDHI treatment can have positive effects on plant chlorophyll content, and on the ratio of chlorophyll to carotenoid pigments, and there were no differences observed between nitrogen levels for these measurements. However, the harvest assessment data showed a trend for differences between nitrogen levels but not between fungicide treatments. There were no interactions between the effects of fungicide and nitrogen level in this experiment. It is therefore difficult to draw conclusions about the combined effect of fungicides and nitrogen levels on the physiology and yields of sugar beet from this experiment. Results did confirm that adding excessive nitrogen is not a suitable solution to improving yields, and that there is an optimum level which should not be exceeded, which reflects the nature of this relationship seen in the literature.

4.4.2 Field experiment

The field experiment had much more favourable conditions in terms of N availability at the beginning of the trial, which was around 20 kg N/ha from a sample of 30 cm depth. This meant that there could be clear set N treatments as low, medium and high, totalling approximately 20 kg N/ha, 60 kg N/ha and 120 kg N/ha of N respectively (combining the 0, 40 and 100 kg N/ha applications with the 20 kg N/ha availability before any applications). These differences were clearest at the beginning of the season, where differences between N treatments were clearly visible by eye. The higher N plants had canopies with higher ground cover than the low N plants, and were noticeably taller too. Later in the season, lower N treatments largely caught up which was reflected in the canopy cover % data calculated from photographs. This suggests that nitrogen availability had more of a significant impact on the earlier growth stages of sugar beet, and less of an influence after these early stages. Using leaf spectral reflectance indices NVDI and NDRE, the leaf cover and health reflected the same relationship as canopy cover % data. This suggested that both the leaf cover and the leaf health were affected by nitrogen levels, especially in the early stages of growth and canopy expansion. The azole + SDHI combination had a positive impact on NDVI and NDRE compared to the no fungicide control, which suggests that this treatment improved both the canopy area and plant health.

In this field experiment, there was good control of disease from all fungicides, and there were no significant differences between any fungicide treatments, with each resulting in lower disease cover than the no fungicide control. This lack of differences between fungicides (other than the control) and the low disease pressure overall provided some confidence that the effects observed in this experiment were due to further physiological effects rather than a result of disease control. Interestingly, nitrogen level did not affect the incidence of rust or powdery mildew, however, there was a trend for higher nitrogen levels to result in higher cercospora incidence (p=0.109), which could indicate that a larger canopy was enough to increase the transfer of infection of cercospora.

The August biomass harvest showed that leaf and petiole weights were significantly higher in the high and medium nitrogen treatment than in the low nitrogen treatment, and total weight followed the same trend. However, by the December harvest these differences were no longer significant. The rankings of the treatments did though match the August harvest, where high N > medium N > low N, which reflected the canopy cover and reflectance data of this experiment. The plants showed large differences in canopy cover, NDRE and NDVI earlier in the season, and became more similar as the season progressed, and the differences in biomass harvests reflected this. These data further suggested that differences in nitrogen levels affect the early growth stages of sugar beet more than they affect the later stages. While there were no significant differences between fungicide treatments in either of the biomass harvests, there was a trend for higher leaf weight in the azole + SDHI treatment and the azole + strobilurin treatment in the August harvest (p=0.079), suggesting that these treatments improved canopy growth at this stage in the season.

At the final machinery harvest, amino nitrogen impurity in the root was higher in the high nitrogen plots than the others, suggesting that although high nitrogen applications can improve yields, the upper limit of application should be carefully decided to avoid negative effects of impurities which can affect sugar extractability. Sugar yield was significantly higher in the high nitrogen plots than the low nitrogen plots, which matches the expected relationship from the literature. The medium nitrogen level was not significantly different from either the high or low nitrogen. The azole + SDHI and the azole treatments resulted in higher sugar yield than the no fungicide control at final harvest, although there was no interaction between fungicide treatment and nitrogen level at this stage.

The impact of insufficient nitrogen on canopy expansion is not limited to the direct result of reduced light interception, but can also be compounded by secondary effects, including the increased light availability for weeds which are competing against the crop. If such weeds are typically outgrown and covered by the crop in a standard growth season, then reduced canopy expansion could lead to the weeds shading the crop and competing for light as well as soil nutrients.

4.4.3 Glasshouse experiment

The glasshouse experiment aimed to join the gaps which were opened during the previous two experiments, including more detailed analysis of plant activity and photosynthesis. The experimental set up in the glasshouse is more favourable for equipment such as the LI-COR, which allowed for regular measurements.

Leaf chlorophyll content (SPAD) was higher in the high nitrogen plants than in the low nitrogen plants, which demonstrated that the low nitrogen was impacting the plant's chlorophyll content. Under the low nitrogen treatment, the fungicide which had higher SPAD values than the others was the azole + SDHI treatment, suggesting that this fungicide may be associated with improved chlorophyll content in low nitrogen conditions. For the leaf photosynthesis measurements, there were no differences found between the fungicide treatments, but differences between the nitrogen levels were clear. For the leaf CO_2 assimilation rate, stomatal conductance, and chlorophyll fluorescence, the high nitrogen plants had higher values than the low nitrogen plants. This subsequently impacted the yield assessments, where high nitrogen plants had higher root weight, leaf weight, and petiole weight than the low nitrogen plants.

The plants in this experiment were treated with fungicides earlier in their growth stages than would be standard for field conditions, due to time constraints with the scheduling of the experiment in year 4 of the PhD project. This meant that plants were not given time to reach the appropriate growth stage and size before fungicide applications were made, and results may have been affected by this. For example, the azole + SDHI treatment had higher leaf SPAD than the other treatments when under low nitrogen, but the azole + SDHI treatment had the lowest root biomass at the end of the experiment. This could be due to the time constraints, where physiological improvements had begun to occur but not for long enough to have an impact on root biomass accumulation. Another reason that this effect might be occurring is that the fungicides may have been applied in too high doses at this early stage of development, which could negatively affect growth. In this case, the azole + SDHI treatment might have had more of a negative impact at this early growth stage than the azole + strobilurin treatment. This highlights the importance of investigating the effect of fungicides on physiology and yields at several stages of growth, and not assuming that any physiological benefits will be found regardless of application timing.

An improvement of stress response may have been the reason that the azole + SDHI treatment had the highest leaf SPAD under low nitrogen conditions, but the lowest overall root biomass. The plants with this treatment might have been prioritising an improved response to stress, where the leaves were protected and remained active, but at the cost of root expansion and yield accumulation. If this experiment had more time, the plants could have been allowed to produce more green area before fungicide application. Additionally, with more time in the experiment the plants could have been left to grow for longer after fungicide application and the knock-on effects of improved stress tolerance may have resulted in improved yields.

4.4.4 Conclusions

Overall, nitrogen level had significant impacts on the physiology and yields of sugar beet plants. In the polytunnel experiment, excessively high nitrogen treatment impacted sugar %, but in the other two experiments, an optimum N rate was achieved and the high nitrogen treatments resulted in better yields than the low nitrogen treatments. This confirmed that nitrogen is a major yield limiting factor, and that it is important for an optimum application to be made.

The field and glasshouse experiments demonstrated that nitrogen availability has a large impact especially earlier in the stages of sugar beet growth. Differences were seen between nitrogen levels for attributes like canopy cover and leaf spectral reflectance indices earlier in the season, and many attributes merged later in the season. This emphasises the importance of early application of nitrogen to sugar beet crops.

In the polytunnel and field experiments, the azole + SDHI treatment frequently outperformed the other treatments. This fungicide had lower CRI and higher NPCI values, indicating that this fungicide resulted in reduced stress, as these indices are indicative of the ratio between chlorophyll and carotenoids. In the field, this fungicide had higher NDVI and NDRE than the other fungicides, which further suggested that this fungicide resulted in plants with a larger and healthier canopy. The azole + SDHI and the azole + strobilurin treatments both resulted in a larger leaf dry weight during the August biomass harvest. At the final harvest, the azole + SDHI and the azole resulted in higher sugar yield than the no fungicide control.

Further research on this topic could compare a larger range of fungicides and their effects on sugar beet plants with nitrogen limitations, with assessments of physiological function and biomass accumulation throughout the growth period.

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Chapter 5 – General discussion and conclusions

5.1 Introduction

In the introduction chapter of this thesis, several research questions and hypotheses were outlined. Each of these research questions informed the design of the experiments in this project, and the relevant results for each of these is outlined below in its own subsection.

5.2 Fungicides influencing chlorophyll, greenness and photosynthesis in healthy plants

The following research question was raised in the introduction chapter of this thesis:

Do certain fungicides increase leaf greenness and other indicators of higher chlorophyll in sugar beet, compared to plants which received no fungicide?

> Hypothesis: One or more of the fungicide treatments will result in higher leaf greenness and other indicators of higher chlorophyll content than the non-fungicide control.

The purpose of this research question was to determine if sugar beet leaves exhibited elevated indicators of chlorophyll content and greenness, similar to the effects which have been observed in other crops. Several studies in other crops have shown particular fungicide treatments resulting in improved yields in healthy plants, with leaf pigment and overall green area index improvements independent of the effects of disease control. This phytotonic effect had not yet been demonstrated in sugar beet before the present study.

In this project, the variables which were used to determine the relationship between fungicides and leaf chlorophyll content and greenness were reflectance indices (including SPAD and RARSb), and green area estimates from canopy photographs.

In the 2021 field experiment, leaf SPAD values were highest for the azole + strobilurin and the azole + SDHI treatments, and lowest for the azole, the strobilurin, and the no fungicide control. In the 2022 field experiment, the azole + strobilurin and the azole + SDHI were the two highest treatments again, however, the range between the lowest and highest values was smaller than in the previous year. In the 2024 glasshouse experiment, there were no significant differences in leaf SPAD between fungicide treatments when nitrogen was sufficient, but, when nitrogen was limited, the azole + SDHI treatment resulted in higher SPAD than the no fungicide control. The no fungicide control was consistently amongst the lowest SPAD values across all experiments, which improves confidence that many of the fungicides were having some positive effects on chlorophyll content in leaves in the presence of very small amounts of disease.

In the nitrogen field experiment (2023), NDVI was higher for the azole + SDHI treatment than the no fungicide control, which suggested that this fungicide improved the green area of sugar beet plants. The same relationship was seen for NDRE, which improves confidence that this effect was true for healthy and active green area rather than just a larger canopy cover. This greening effect appeared to be functional, as the azole + SDHI treatment resulted in a higher sugar yield than the no fungicide control in that same experiment. Interestingly, in the 2021 field experiment, the azole + strobilurin had the highest NDRE values, and the azole + SDHI was not significantly higher than the no fungicide control. At the end of that experiment, the azole + strobilurin combination had the highest sugar yields, which is consistent with the high NDRE values, but inconsistent with the 2023 experiment. This suggested that seasonal factors affected the activity of the fungicides, and the optimal conditions for their benefits. Additionally, the variety of sugar beet used was different in these experiments, so this may suggest that the fungicide's physiological effects could vary depending on the variety used. For this reason, it would be valuable for future research to investigate the effects of fungicides on several varieties of sugar beet from several different breeders.

In this project there were significant differences in leaf CO_2 assimilation, stomatal conductance, and chlorophyll fluorescence between nitrogen levels, but there were no differences observed between fungicide treatments. It may have been expected to find that these measurements were higher in plants treated with fungicides than in those without. A potential reason for the lack of this effect could have been that the plants were still small in size at the timing of fungicide application due to time constraints, and this application may have been too early in the plant growth process to be useful for physiological effects. It would be useful in future research to collect data on these traits with a later application of fungicide, to test if any physiological benefits appeared at that stage.

The relationship of improved chlorophyll and photosynthesis is seen in the literature for other crops. Jacobelis et al. (2023) found that tomato plants treated with fungicides had increased CO_2 assimilation, higher chlorophyll a and b, and increased activity of the antioxidant system, which resulted in higher production. The study noted the strobilurin as the treatment which had the most substantial effect on these physiological improvements. Similarly, another study on tomato plants found that each fungicide included in the study improved photosynthetic activity, particularly those which contained both a strobilurin and an SDHI, which subsequently resulted in the highest yields (Marek et al., 2018).

For this reason, conclusions can be formed about the greenness of plants treated with various fungicides, however, more research is required to form a clear conclusion about the specific photosynthetic processes between fungicide treatments. Focusing solely on leaf greenness and healthy canopy area, the fungicides which showed positive responses compared to no fungicide control were both the azole + strobilurin and the azole + SDHI treatments. Further research could use the LICOR equipment in field conditions on plants treated at the appropriate timing for their growth stage, to determine if these fungicides performed well in these measurements too.

5.3 Delayed senescence and stay-green effect of fungicides

The following research question was raised in the introduction chapter of this thesis:

If leaf greenness is increased by fungicide treatment, does this greenness continue later into the season, as to represent delayed senescence?

> Hypothesis: Leaf greenness will be higher than the non-fungicide controls later into the season.

The purpose of this research question was to investigate the concept of stay-green effect in sugar beet plants. This relationship has been demonstrated in other crops, where fungicides have been shown to delay senescence of leaves, allowing the plant to continue photosynthesising later into the season and accumulate higher yields. This relationship has been suggested in sugar beet, where growers have noticed plant remaining greener for longer in the season with fungicide use, but this has not yet been recorded in experimental conditions.

In the 2021 field experiment, canopy senescence was faster in the no fungicide control and the azole + SDHI treatment than in the other fungicides, with a significant interaction between fungicide treatment and time. This demonstrated that the other fungicides were more effective at maintaining the canopy area towards the later end of the season. In the same year, NDRE and NDVI were highest for the azole + strobilurin treatment, and lowest for the no fungicide control and the azole + SDHI treatment.

Strobilurins in particular delaying leaf senescence is a common trend in the literature. Ruske et al. (2003) found that adding strobilurins to azole-only fungicide programs in winter wheat delayed the flag leaf senescence, and yields were improved. This suggested not only that the fungicides can delay senescence but that the increased green leaf period was functional and contributed to further yield accumulation. Ijaz and Honermeier (2012) studied the effect of fungicides on winter rapeseed and found that the azole + strobilurin combinations resulted in delayed senescence and improved yields compared to untreated control. Ido et al. (2020) studied the effect of a strobilurin treatment (mandestrobin) on a selection of plants, and observed delayed chlorophyll degradation in *Brassica napus* plants, which is part of senescence. The authors found that there had been a downregulation in chlorophyll degradation genes as a result of the fungicide treatment.

Strobilurins are not the only fungicide group to have been observed to show this effect. Berdugo et al. (2013) used a range of sensors and imaging techniques to gain a detailed understanding of how different fungicides affect senescence in wheat plants, and found that all of the selected fungicides delayed senescence and extended the green leaf area duration. Specifically, the results showed that all fungicides in the experiment improved chlorophyll fluorescence and thermal responses, but the SDHI treatment (Bixafen) performed better in measurements of leaf reflectance, spectral indices, and water content than the azole and the strobilurin. Interestingly, although the SDHI treatment performed better than other fungicides in the physiological measurements, the higher yields were actually associated with the azole treatment and the strobilurin treatment. This could suggest that the SDHI improved leaf health, and delayed senescence effectively, but that these improvements were not enough to improve yield accumulation in the disease-free conditions of the experiment.

Wu and von Tiedemann (2001) investigated the effects of azoxystrobin and epoxiconazole on wheat senescence, and found that both fungicides significantly delayed senescence, and this was associated with an increase in antioxidant processes which decreased harmful active oxygen species. This study showed that ethylene may not be the primary method by which these fungicides interact with the senescence process, which was previously suggested.

Cromey et al. (2004) investigated the effects of azoxystrobin and tebuconazole and observed that both fungicides maintained green leaf area for longer than the untreated plots. Tebuconazole increased yields in almost every trial, and azoxystrobin increased yields in every trial, suggesting that while both treatments were highly effective, azoxystrobin may have had more powerful physiological benefits in the conditions of the experiment. Conversely, Blandino and Reyneri (2009) investigated the effects of azoles and strobilurins on winter wheat and found that while the azole delayed senescence, this did not increase the grain yield, and adding a strobilurin to the treatment didn’t further delay senescence nor increase grain yield compared to the azole alone. This suggests that a fungicide-induced delay in senescence may not always result in functional responses, and yields may not always increase as a result of longer green area.

In the field experiments in this project, the azole + strobilurin combination was frequently associated with higher SPAD values, NDVI and NDRE compared with no fungicide control, and this continued late into the season. In the 2021 field experiment, the azole + SDHI was observed to have a similarly high SPAD later in the season, however, in 2022, this treatment did not perform as well as the azole + strobilurin. In the 2021 season, the high leaf greenness measurements associated with the azole + strobilurin resulted in higher yields too, suggesting that this increased greenness was functional, and that the extended maintenance of greenness was beneficial to yield production. Interestingly, in the 2021 season the no fungicide control had similar SPAD values to other treatments for much of the season, but the vales began to reduce heavily as the season went on, while many of the other treatments maintained their SPAD values. This suggests that the major impact of improved greenness was the lengthened maintenance of the canopy, rather than increased greenness throughout the entire season. This is particularly relevant for sugar beet, which can continue to accumulate yields late in the season, and with flexible harvest times which may extend far into the year. These data showed that the combination of azole + strobilurin was more effective at improving green area, particularly later in the season, compared to the azole or strobilurin acting alone. This mirrored the relationship which was found in the study by Ruske et al. (2003), where adding a strobilurin to an azole-only program was more effective at delaying senescence than the azole alone. It could be speculated that strobilurin treatments are more effective at inhibiting ethylene formation and increasing cytokinin levels than azole-only treatments, resulting in more effective delayed senescence. As the azole + SDHI treatment in the present study also displayed improved greenness in some of the experiments, it could be suggested that the SDHI treatments may have a similar mechanisms of delaying senescence as the strobilurin treatments. It would be useful to compare the effects of strobilurin treatments and SDHI treatments at the hormonal level of stress responses during senescence to improve the understanding of these effects.

5.4 Effects of fungicides on varieties with different disease susceptibility

The following research question was raised in the introduction chapter of this thesis:

Do the positive effects of fungicides occur similarly in disease susceptible varieties and disease resistant varieties?

> Hypothesis: Positive effects of fungicides will be observed in both the disease susceptible and disease resistant varieties, improving confidence that effects are not exclusively due to pre-symptomatic disease control.

One of the main concepts considered in this project was the extent to which physiological improvements are a result of early disease control rather than further effects of fungicides. To provide a useful comparison, this project included two varieties of sugar beet with differing disease susceptibility. The aim was to compare all effects of the fungicides between a disease susceptible variety and a disease resistant variety. If the physiological benefits only seemed to occur in the disease susceptible variety, it would have suggested that the effects seen are simply a knock-on effect of early disease control.

The two varieties included were Kortessa (low disease susceptibility) and Advena (high disease susceptibility) from the same breeder (KWS). While there were some differences observed between the varieties for some physiological trait measurements, such as Triangular Vegetation Index and canopy cover, there was generally no interaction between variety and fungicide, indicating that the effects of fungicides were consistent across both varieties. This improved confidence that the effects observed were not exclusively due to disease control.

Advena had significantly higher incidence of rust infection than Kortessa (up to 20% canopy cover of Rust with Advena compared to less than 5% with Kortessa, in the no fungicide control in 2021), which was expected. Interestingly, Advena had significantly higher canopy cover than Kortessa, suggesting that this variety had better canopy expansion. It is possible that Advena may have had a more prostrate canopy architecture and therefore appeared larger than if it had upright architecture when photographed from above, but canopy height was not measured in this experiment so this could not be confirmed.

The purpose of including two varieties with different disease susceptibility was to ensure that there could be some confidence that any physiological effects which occurred were not due to early disease control alone. As the fungicides generally performed equally across both varieties, there is good evidence that the benefits observed in this project were at least partially due to factors aside from disease control. This has also been observed in the study of Bingham et al. (2021) which compared the responses of spring barley plants treated with different fungicides, and found that the protectant fungicide chlorothalonil controlled disease to an equal level as prothioconazole and pyraclostrobin, but it did not result in the physiological benefits of the latter treatments. This demonstrated that while these products do control disease, the beneficial effects observed are not exclusive to the effects of disease control.

5.5 Drought stress resilience by fungicide use

The following research question was raised in the introduction chapter of this thesis:

Do sugar beet plants have more resilience against the effects of drought stress if they have been treated with fungicides?

> Hypothesis: Sugar beet plants will have more resilience against the effects of drought stress if they have been treated with fungicides.

Much of the literature on the topic of physiological effects of fungicides found that there was an interaction between fungicides and stress conditions. Of the types of stress explored in the literature, water availability was amongst the most common, where studies have shown crops having increased resilience to the impacts of drought when treated with fungicides. This project aimed to determine if the relationship between fungicides and drought stress tolerance was also apparent in sugar beet.

While there were some clear effects seen between fungicides and the no-fungicide control in this experiment, these only occurred in well-watered conditions for the majority of traits. For most of the results, there were no differences observed between fungicides under drought, which was not expected. One instance of a trend in this experiment was that leaf canopy temperature appeared to be lower in droughted plants that had received fungicide than in those which did not receive any. This trend indicated that the fungicide-treated plants may have been able to maintain transpiration during drought more effectively than the no-fungicide controls, allowing the leaves to stay cooler. This is indicative of an altered stress response, where the plant may typically respond to drought by closing stomata and retaining as much available water as possible; the fungicides may have changed the response of the plants to allow many plant processes to continue.

There was an interaction between fungicide and irrigation for leaf area, where there was a larger difference in the leaf area of well-watered vs droughted plants when they had received no fungicide, suggesting that there had been some beneficial effect under fungicide treatment.

There was also a trend for the leaf greenness (SPAD) values to be higher in the strobilurin treatment under droughted conditions, but the strobilurin treatment resulted in the lowest SPAD in well-watered conditions. While this suggested that the strobilurin may have been helping the plants to retain their chlorophyll during stress, there is a possibility that the leaves were actually smaller and more compact due to the drought, and this resulted in the higher values. This can occur when the components within the leaf become more concentrated.

When comparing the gas-exchange data, there was a trend for CO_2 assimilation and stomatal conductance to be higher in both of the fungicide treatments compared to the untreated control in well-watered conditions. This suggested that these fungicides improved the overall photosynthetic activity of plants when conditions were favourable, however the effect was much less pronounced under droughted conditions, suggesting that drought may have limited the plants more than the fungicides were capable of mitigating. There was a trend for stomatal conductance to be higher in the SDHI, followed by the strobilurin, and then the untreated control, which suggests that the SDHI may have been more effective at keeping stomatal movement optimal, although this trend was diminished under droughted conditions, further emphasising that the fungicides may not have been effective enough to reduce the effects of the drought response in this case.

In the literature, several studies have noted the effects of fungicides on plant hormones including ABA, cytokinins, and ethylene. The trend was typically that the hormones associated with preserving resources or shutting down were decreased, and the hormones associated with increased activity were increased. While this may seem counterintuitive to survival during stress conditions, these responses may allow for the plant to continue photosynthetic processes and yield formation in conditions that would normally cause the plant to slow down productivity. If stress is long term, these effects may become unhelpful, but for the example of drought stress in the UK, periods of drought tend to be short term, allowing for plant recovery afterwards. For this reason, a temporary reduction in the plants response to stress may be beneficial in areas where stress is short term.

Additionally to the changes in plant hormone activity, recent studies have demonstrated that sugar beet are able to continue photosynthesising in droughted conditions, notably longer than many other crops. Even in the absence of fungicides, sugar beet are able to retain physiological activity and keep stomata open during drought, allowing them to continue photosynthesis (Barratt et al., 2023). This response to drought compared with other crops, which are more prone to closing stomata and retaining resources during drought, may be one of the reasons that the relationship observed in this study did not appear to mirror the relationship seen in other crops.

While the effect of fungicides on these particular plant hormones has been studied in other crops, further research on the hormonal response in sugar beet specifically and the subsequent effects on stress response would improve understanding of this topic.

5.6 Fungicides mitigating effects of insufficient nitrogen

The following research question was raised in the introduction chapter of this thesis:

Do fungicides alleviate the negative effects of insufficient nitrogen availability on sugar beet growth and yield?

> Hypothesis: The effects of insufficient nitrogen availability will be more severe in plants which received no fungicide than in plants which received a fungicide.

Fungicides have been shown to improve the ability of other crops to cope with insufficient nitrogen, but this relationship had not yet been explored in sugar beet before the present study. This project aimed to compare the response of sugar beet plants provided with a range of nitrogen fertiliser doses and a selection of fungicide treatments. The aim was to determine if fungicides could alleviate the negative effects seen in sugar beet grown in soil with insufficient nitrogen.

In the polytunnel trial, where the baseline available nitrogen was already high, adding nitrogen fertiliser had a negative effect on sugar %. In the field experiment there were clear differences between nitrogen treatments, especially earlier in the season. In the glasshouse experiment, time constraints resulted in fungicides being applied earlier than standard to the plants. This potentially reduced the effects of the fungicides and their impact on plants with insufficient nitrogen.

In the polytunnel experiment, the SDHI and the azole + SDHI treatments had higher Blue Green Index, which suggested that they had improved chlorophyll compared with the other fungicide treatments. The azole + SDHI treatment also indicated a good level of chlorophyll in the glasshouse experiment, whereas under low nitrogen conditions, this fungicide has higher SPAD values than the other fungicides. In the field experiment, the azole + SDHI treatment resulted in higher NDVI and NDRE treatments than the no fungicide control, which suggested that this treatment improved the plant canopy and capacity for light interception. Providing an insight into potential stress reduction, NPCI values represent a ratio of chlorophyll to carotenoids. The azole + SDHI and the SDHI treatments had the most favourable NPCI measurements, which indicated that these treatments resulted in plants which underwent less stress than in the other treatments. With these results, there is a strong indication that fungicides containing an SDHI have particularly beneficial effects on plants which are grown in conditions with insufficient nitrogen.

Biomass harvests were used in the field experiment for this chapter, in order to understand the production and partitioning of biomass through the season. From the August biomass harvest, there were differences between nitrogen levels for several measurements; the leaf, petiole and root dry weights were all higher in the high and medium nitrogen levels compared to the low nitrogen. This response mirrored what had been seen in other physiological factors like leaf area, where the high and medium nitrogen levels appeared more similar to each other than the low nitrogen level. However, later in the season this relationship was no longer significant, and differences between nitrogen levels were much smaller. This suggests that plants with lower nitrogen were able to catch up after the slower start to leaf expansion, perhaps because the soil became more enriched through the season due to N mineralisation and differences in available nitrogen were reduced. At the final machine harvest, sugar yield was significantly higher in the high nitrogen plots than the low nitrogen plots, so it might be the case that root expansion was similar at the end of the season between nitrogen levels, but that the sugar production and storage was more effective in conditions with sufficient nitrogen availability. It may also be the case that the sugar production was more affected by the differences in canopy cover and subsequent light interception early in the season, and that although biomass production caught up later in the season for all nitrogen levels, this was too late to produce the same sugar yields. This places importance on the process of reaching canopy closure as early in the season as possible.

When comparing leaf SPAD values from the glasshouse experiment, the high nitrogen treatment resulted in higher SPAD values than the low nitrogen treatment, which was expected. This suggests that in the conditions of the experiment, insufficient nitrogen resulted in reduced chlorophyll in leaves. Under low nitrogen conditions, the azole + strobilurin treatment was not significantly different from the low nitrogen and no fungicide control, which may suggest that the azole + strobilurin was not able to mitigate the effects of insufficient nitrogen. In these low nitrogen conditions, the SPAD values of azole + SDHI treated plants were significantly higher than the other fungicides, indicating that this fungicide had some ability to reduce the negative effects of insufficient nitrogen on chlorophyll content.

The leaf gas-exchange results showed that high nitrogen plants had significantly higher CO_2 assimilation rates than low nitrogen plants, which was the expected relationship. This highlighted the importance of sufficient nitrogen for photosynthetic activity. The same relationship was seen for stomatal conductance, demonstrating that sufficient nitrogen is vital for optimal gas exchange and stomatal movement. The relationship was also seen when comparing chlorophyll fluorescence. For each of these traits, the nitrogen and fungicide interaction was not significant, suggesting that nitrogen availability has a much higher rate limiting effect than the fungicides could mitigate.

The harvest results for the glasshouse experiment showed large differences between the high nitrogen and low nitrogen treatments. High nitrogen plants had much larger roots than the low nitrogen plants, demonstrating the limiting effects that nitrogen insufficiency can have on root expansion. There was a trend for the azole + SDHI treated plants to have lower root weight than the untreated control and the azole + strobilurin plants. The azole + SDHI treatment performed well in other experiments of this project, so for it to perform lower than the untreated control here was not expected. A possible cause for this result could be that the product had negative effects on root expansion due to the early stage of fungicide application. Normally the plants would be much larger at the time of treatment, but due to time constraints the plants were treated at a relatively small size, and the fungicide may have interacted differently with the plant than it would have if treated at a standard timing. Further research in this area would be useful, to compare how these fungicides interact with the plant at a range of treatment times earlier than standard, to determine if any negative effects are consistently seen. An additional reason that the azole + SDHI treatment may have performed well physiologically but yielded poorly in this experiment could be that the plant may have been responding well to stress conditions, but at the expense of root expansion. Repeating this experiment with a more optimal treatment timing would determine the true relationship between the selected fungicides and their response to a range of nitrogen levels.

Although not statistically significant, there was a trend for cercospora infection to be higher in the high nitrogen plants than in the low nitrogen plants during the field experiment. This could be a result of a larger canopy area increasing the contact between infected plants, or more availability of nitrogen in the leaves of plants which the pathogen requires to survive and reproduce. Each of the fungicides in the present study reduced infection regardless of nitrogen level.

5.7 Differences in effects between fungicide groups

The following research question was raised in the introduction chapter of this thesis:

Do all fungicide groups result in the same physiological effects, aside from disease control, or do some fungicide groups have different physiological effects to others?

> Hypothesis: There will be differences between the physiological and yield effects of some of the fungicide groups.

One of the aims of this project was to determine if physiological effects differed significantly between fungicides, rather than only finding a difference between fungicides and non-treated controls. While the different fungicide groups have different modes of action, the difference between their physiological impacts has not yet been explored in sugar beet. This section draws upon results from each of the above sections to determine any differences between the activity of the fungicides used in this project. For much of this project, combinations of fungicide groups were used (namely azole + strobilurin and azole + SDHI), to replicate the type of fungicide products which are routinely used on UK crops.

In the drought experiment, the SDHI and the strobilurin performed similarly to one another in well-watered and droughted conditions, both usually outperforming the no fungicide control. One measurement where they differed significantly was leaf SPAD, where the strobilurin had the lowest values compared to the SDHI and no fungicide control under well-watered conditions, but had the higher SPAD values under droughted conditions. Wilted leaves can result in high SPAD values so it's difficult to draw meaningful conclusions about this but it is interesting that the two fungicides differed in their response to drought. In the same experiment, the SDHI treatment resulted in a larger leaf area than the strobilurin and the no fungicide control under well-watered conditions, but all fungicides performed similarly under the droughted conditions (including the no fungicide control). This suggested that while the SDHI may have been more effective at improving canopy expansion, it may not have been an effective mitigation for the effects of drought in sugar beet. Interestingly, when it came to yields, the strobilurin had the lowest root weight out of all treatments, including the no fungicide control. This experiment showed the SDHI treatment to have more favourable values for most of the parameters.

In the variety field experiment, the two combination fungicides (azole + strobilurin and azole + SDHI) had higher SPAD values than the other fungicides in 2021. In the 2022 experiment, the azole + strobilurin had the highest SPAD again, but the azole + SDHI treatment was no longer significantly higher than the no fungicide control. Interestingly, in 2021 the NDRE values of plants treated with azole + strobilurin were the highest of all treatments, and the azole + SDHI treatment was not significantly higher than the no fungicide control. The combination of high SPAD with lower NDRE for the azole + SDHI treatment might suggest that these plants had a lower canopy cover %, but the individual leaves were healthier and greener than other treatments. In the 2021 experiment, the azole + strobilurin treatment had the highest specific leaf weight, and also had the highest sugar yield, suggesting that this treatment improved the health and activity of leaves and subsequently increased photosynthesis and yield production. For the above reasons, the combination fungicides (azole + strobilurin and azole + SDHI) were the better performing fungicides, but in particular the azole + strobilurin had consistently higher measurements for leaf health and resulting yields.

In the nitrogen experiments, the azole + SDHI combination appeared to be more effective at reducing the stress of plants, for example, showing lower values of carotenoid reflectance index than the other fungicides in the polytunnel trial. In the field experiment, the azole + SDHI treatment had higher NDVI and NDRE values than the no fungicide control, where the other fungicides did not differ significantly from the control. This suggested that this treatment was most effective at keeping a large and healthy canopy. In the same experiment, both the azole + SDHI and the azole + strobilurin had a trend for a higher leaf weight than the no fungicide control in the first biomass harvest, however by the winter biomass harvest these differences had disappeared. This suggested that the fungicides may have a large influence on earlier canopy expansion and growth, but less of an impact on later canopy expansion. There was a trend for the azole + SDHI treatment to result in a larger root weight in the winter harvest than the azole + strobilurin combination. For most of this set of experiments, the azole + SDHI treatment was the best performing treatment. In the glasshouse experiment, the azole + SDHI treatment had higher SPAD values than the no fungicide control in low nitrogen conditions, but this was not the case when nitrogen was sufficient. At harvest, there was a trend for the azole + SDHI treatment to result in lower root weight than the no fungicide control, although this was not significant.

Taking all of the experiments into account, it is clear that the azole + strobilurin combination tended to have highest performance in physiological traits and in yields, closely followed by the azole + SDHI treatment which often performed best in physiological traits, but had varying effects on yields.

5.8 Future research

The topics explored in this research open up new research questions, and areas for more detail to be determined. One of the limitations in this project was that a selection of fungicides and varieties had to be chosen to compare, which could not be too large to record sufficient data. With fewer limitations on size of the experiment, it would be useful to compare several products within each fungicide group, rather than one of each. Not all fungicides from the same group have the same effect on plants, and it's difficult to draw confident conclusions based on how one product from each group performed. For one year of this experiment, two products both belonging to the 'azole + strobilurin' group were used, and they did not have identical results. A comprehensive study where at least two products from each group & each combination were used would provide a better understanding of how these products interact with the crop. In a similar way, the main varieties used in this project were Kortessa, Advena and BTS1915, however it would be useful to compare how these fungicides affected plants from each of the varieties on the BBRO recommended list. Some varieties become less commonly used by growers within years of appearing on the list and it would improve confidence in conclusions to know whether the fungicides affect a large number of varieties in the same way. Additionally, it would be useful to repeat the experiments from this study in a selection of areas in the UK with differing soil types and weather conditions, to quantify the effects in different sites and seasons.

Another area where future research could focus is the specific hormonal responses to fungicide application, and subsequent hormonal changes associated with stress responses. The topic of altered stress responses appeared to be relevant within several parts of this project, and it would be useful to compare the hormonal responses of sugar beet plants treated with fungicides in a range of conditions, such as the timing of senescence, drought stress, and nitrogen insufficiency. This project focused on physiological responses in the canopy and yield formation, but a more detailed insight to these hormonal changes would be useful. These changes have been explored in other crops and it would be valuable to compare these against sugar beet, particularly for contexts such as drought stress, where sugar beet has already been shown to respond differently to drought than many other crops.

Leaf samples would need to be collected and frozen before analysis to avoid degradation, and hormones would need to be measured by liquid chromatography-mass spectrometry (LC-MS).

In this context of comparing response to stress, Ozaki et al. (2023) demonstrated that rice plants grown in low nitrogen conditions had increased non-photochemical energy dissipation in photosystem I. Non-photochemical energy dissipation is the process by which excess light energy that isn't used during photosynthesis is dissipated to prevent damage to the photosystem apparatus. In conditions of stress, non-photochemical energy dissipation can increase due to an inability for the photosystem to receive electrons at a normal rate, leading to a risk of energy accumulation which can damage the photosystem apparatus. In non-stressed conditions, non-photochemical energy dissipation would be expected to be low, meaning that the process is running efficiently and there are no limitations leading to energy accumulation, and that most of the light energy being absorbed is being used for productivity. With this in mind, the study by Ozaki et al. (2023) showed that the activity of the photosystems is directly affected by nitrogen deficiency. The study puts forward the explanation that low nitrogen conditions lead to low Rubisco amounts, which subsequently reduces leaf CO_2 assimilation rates. This reduction in CO_2 assimilation can result in the supply of NADPH and ATP from the photosynthetic electron transport exceeding the demands of the photosystem apparatus, leading to energy accumulation. This concept could be a useful topic to explore in further research of this relationship, as the photosystem response to low nitrogen could be compared between multiple fungicides to determine the nature of the altered stress response.

5.9 Conclusions

To conclude, this study found that while fungicides tended to perform better than the no fungicide control in a range of conditions, their response to drought was inconclusive, which may have been due to sugar beet's ability to cope with stress better than many other crops. In field conditions, this study was able to demonstrate that the combinations of fungicides (azole + strobilurin and azole + SDHI) tended to have better physiological performance than other fungicides and the no fungicide control, and sugar yields tended to be highest for the azole + strobilurin combination. There appeared to be a positive relationship between specific leaf weight and yields, suggesting that fungicides which improved leaf thickness may have been more effective at improving yields. In the nitrogen experiments, the azole + SDHI appeared to allow plants to reduce stress responses, and had better physiological performance than other fungicides early in the season, however, this did not consistently result in improved yields.

This study demonstrated that particular fungicide groups interact with sugar beet physiology differently, and that the effects on the plants are complex and there are several physiological processes involved in the determination of yield performance. Overall, the combination fungicides (azole + strobilurin and azole + SDHI) were particularly effective at improving physiology, and the azole + strobilurin in particular had a clear positive impact on yield development.

There is some indication from the current study that the fungicides may have been interacting with the stress response of sugar beet plants, and allowing them to improve their physiology and yield formation in a range of conditions. This was demonstrated through factors such as delayed senescence, canopy NDRE, and better chlorophyll : carotenoid reflectance ratios. This would particularly apply under stress conditions, which caused accelerated senescence late in the season, which would align with the ‘stay green effect’ which has been observed. In addition to allowing the plants to continue photosynthesis in conditions of stress, this effect may have allowed the plants to continue photosynthesis in a more subtle range of conditions which would normally slow down activity, aside from measurable stress.

If this reduced stress response is a major part of the relationship in this project, this would align with the research which showed that sugar beet has a less severe reaction to drought on a plant hormone level, and are able to continue photosynthesis in dry conditions. If sugar beet already demonstrate a less severe reaction to drought than other crops, and fungicides interact with this stress response, it would be expected that this relationship would not be the same in sugar beet as in other crops.

More research is required to determine the effects of these fungicides on more detailed plant processes. For example, where this study used indicators of stress such as reduced NDRE or increased carotenoid reflectance index, it would be valuable to clarify further these responses by observing the hormones involved in plant stress responses. Additionally, more detailed measurements about leaf photosynthetic activity resulting from the contrasting nitrogen levels and fungicide applications would be useful, as this study was limited due to time constraints by only recording leaf photosynthetic traits at specific stages during the glasshouse experiment.

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