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Improving resource use efficiency in barley, through protecting sink capacity.

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1. Abstract

The aim of this project was to identify how fungicides increase the resource use efficiency and yield of spring barley so that fungicide strategies can be tailored more effectively to account for the disease resistance of the variety and the potential physiological response of the crop. Specifically, three broad research questions were addressed which have scientific and commercial relevance. 1) What duration of protection of canopy light interception is required post-anthesis to maximise yield? 2) How do fungicides increase yield where visible disease severity is low or absent? 3) How should fungicides be timed in low disease risk situations?

Detailed physiological measurements were made in experiments conducted by research partners at two main sites, ADAS in Herefordshire and SRUC in Edinburgh. Experiments by industry partners tested the validity of the findings over a wider range of varieties and sites differing in disease pressure. Results showed that light interception by the canopy must be protected for approximately the first 75% of grain filling in order to maximise yield; a period of 3–5 weeks from 50% ear emergence depending on the site and year. After that period yield is insensitive to major reductions in light interception, probably because grain filling can be completed using dry matter from storage reserves. Treatment of disease-susceptible varieties with prothioconazole plus pyraclostrobin (products Proline and Comet 200, respectively) at the start of stem extension gave adequate protection of the canopy over the critical first 75% of grain filling when disease pressure was low. Under higher disease pressure an additional treatment during booting was needed. Although later applications after ear emergence protected the canopy for longer, they had no effect on yield because the additional protection occurred late in, or after, the critical period.

Field experiments over a wide range of varieties and sites showed that, on average, yield responses in the order of 0.3–0.4 t ha^{-1} were obtained from treatment with Proline and Comet in the absence of visible disease. The yield increases were largely the result of an increase in the number of grains produced m^{-2}. A comparison of the effects of Proline and Comet with that of chlorothalonil (product Bravo 500) indicated that the grain number response was not the result of the control of visible disease, the control of symptomless pathogen infection and leaf saprophytes, or a delay in leaf senescence. It appeared to result from a direct effect on plant metabolism which occurred before flowering. A single application during booting was sufficient to elicit the response. The results have implications for fungicide treatments in low disease risk situations, e.g. where resistant varieties are grown or where the disease pressure is low. If there is no disease present at the start of stem extension, fungicide treatment can be withheld. However, an application of prothioconazole plus pyraclostrobin at booting can be justified economically as it will provide insurance against late season disease and will result in yield enhancement, even if disease fails to develop. It can be further justified in terms of improvements in N use efficiency and reduced greenhouse gas emissions per tonne of grain yield.

2. Introduction

Spring barley is a valuable component of cropping systems. It provides specific conservation and wildlife benefits over winter crops. For example, overwinter stubble affords more foraging and nesting opportunities for farmland birds. Barley production in general also aids effective land use as it can be grown at marginal sites less suitable for the production of other arable crops and is an important component of good rotational practice. However, for barley production to be economically and environmentally sustainable, high yields of quality grain need to be obtained consistently with the minimum of inputs.

Foliar disease reduces the efficiency with which crops use water and energy. Disease can affect plant water relations through effects on root growth, the integrity of the leaf cuticle and stomatal regulation (Ayres, 1981; Walters, 1985; Prats *et al.,* 2006; Grimmer *et al*., 2012). Disease decreases energy efficiency by reducing the dry matter produced per unit of energy expended in crop husbandry (Berry *et al.*, 2008). As fertilizer nitrogen (N) accounts for approximately half the total energy input into arable production, management of disease to maximise yield per unit of fertilizer N applied is essential to maximise the energy efficiency of barley production. However, control of disease with fungicides needs to be targeted only at those crops likely to give a significant response in terms of increased grain yield or quality, in order to minimise any environmental impacts and selective pressure for fungicide resistance (Bingham *et al.,* 2012a) and for economic reasons. Identifying potentially responsive crops requires an understanding of how disease influences the yield forming process and the effects of fungicides on both the pathogen and plant.

Barley yield in the UK is predominantly sink-limited. Yield is determined by the number of grains produced and their capacity to store assimilates, rather than the crop's ability to provide assimilates to fill potential storage (Bingham *et al*., 2007a & b). The number of grains is determined before flowering by the production and survival of tillers and spikelets; processes that are sensitive to variation in light interception (Arisnabarreta and Miralles, 2008). The potential size (storage capacity) of grains is believed to be set by the development of the ovary pre-flowering and the grain endosperm early post-flowering (Bingham *et al.,* 2007b; Hasan *et al.,* 2011).

Pathogens may reduce crop growth by reducing radiation interception, radiation use efficiency (biomass production per unit of radiation interception by healthy green tissue) and the partitioning of assimilates (Boote *et al*., 1983; Johnson, 1987; Gaunt, 1995). This, in turn, may reduce yield by restricting the formation of each of the major yield components (Gaunt, 1995). Early epidemics of foliar pathogens which develop during canopy expansion may reduce the number of ears produced

and the number of grains per ear because disease infection coincides with the period of tiller and spikelet production and survival (Brooks, 1972; Lim and Gaunt, 1986; Conry and Dunne, 1993). A key fungicide timing in winter barley is at the start of stem extension as this maximises tiller and spikelet survival and hence the formation of grain sites. Later applications just prior to ear emergence (i.e. during booting) often give smaller additional increases in yield through an increase in average grain weight (Bingham *et al*., 2010). In spring barley, the stem extension and booting application timings generally result in more comparable yield responses depending on the nature of the disease epidemic (Bingham *et al.,* 2010).

A widely held view within the industry is that late season (post-anthesis) disease reduces average grain weight through effects on the availability of assimilate for grain filling and hence disease management should seek to maximise the duration of canopy green area post-flowering (as in wheat). However, recent evidence suggests that fungicides applied just prior to ear emergence increase average grain weight predominantly by increasing potential grain size rather than assimilate availability for grain filling. Average grain weight was largely unaffected by agronomic treatments designed to vary the amount of post-anthesis assimilate per unit grain number, but was increased by fungicide in both winter and spring barley (Bingham *et al.,* 2010). Since potential grain size is determined over a relatively short period of time either side of flowering, the practical implication of these findings is that protecting green leaf area late into the grain filling period may be unnecessary. However, this hypothesis required testing, as the point at which green area light interception can be reduced without affecting yield was not known.

Thus, in contrast to wheat where the objective of disease management is to protect the post-flowering production of assimilate for grain filling, the primary aim of disease management in barley is to protect the development of sink capacity (Bingham *et al*., 2010; HGCA, 2013a). However, it has also been demonstrated that yield responses to fungicide in barley are variable and do not relate well to the amount of visible disease present, which suggests that fungicides may influence the development of sink capacity in ways other than through the control of visible disease (Bingham *et al.*, 2010; Bingham *et al*., 2012a). Treatment of low, or sometimes apparently nil, disease can result in substantial increases in grains per m^2, and hence in sink capacity and yield (Bingham *et al.,* 2012a). Visual assessment of disease is subjective, but the extent of the discrepancy between disease severity and yield is too large to be attributed to assessment error. This has important implications for the rational use of fungicides, because it means that the requirement for fungicide treatment, expressed in terms of likely improvement in yield or quality, cannot be predicted just from an assessment of the amount of visible disease present in the crop, or the risk of a disease epidemic developing.

There are several possible mechanisms that might account for yield responses to fungicide where there is little or no visible disease. Firstly, grain number formation may be particularly sensitive to low levels of disease that have a relatively small impact on canopy light interception because of symptom location low in the canopy. Secondly, fungicide treatment may be controlling symptomless pathogen infection. Molecular, microscopic and serological techniques have identified fungal infection in the absence of symptom development in a number of pathosystems including *Rhynchosporium commune* and *Ramularia collo-cygni* of barley (Fountaine et al., 2007; Walters *et al.*, 2008; Sowley *et al.,* 2010; Thirugnanasambandam *et al*., 2011). The fungus may grow systemically within plant tissues before visible symptoms develop, but the impact of this symptomless phase on crop growth and yield formation has not been tested previously (Walters *et al.,* 2008). A third possibility is that fungicides have direct effects on grain sink capacity and yield by modifying plant metabolism or assimilate partitioning. Some triazoles have been reported to impair gibberellin biosynthesis (Rademacher, 2000), which could conceivably increase grain numbers. In wheat, triazole and strobilurin fungicides have been found to delay leaf senescence in the absence of visible disease and the prolonged canopy lifespan is correlated with an increase in yield (Wu and von Tiedemann, 2001; Cromey *et al*., 2004). It has been suggested that the strobilurins may delay senescence by reducing ethylene production and the rate of cytokinin degradation (Grossman *et al.,* 1999), although other lines of evidence suggest that triazoles and strobilurins delay leaf senescence by reducing oxidative stress (Wu and Tiedemann, 2001). In addition, control of saprophytic fungi on the leaf surface has been implicated in the yield response to fungicide in the absence of visible disease (Smedegaard-Petersen and Tolstrup, 1985; Bertelsen *et al.*, 2009). Saprophytes may reduce yield by decreasing leaf lifespan and increasing metabolic costs associated with defence reactions to unsuccessful infection attempts by the fungus (Smedegaard-Petersen and Tolstrup, 1985; Bertelsen *et al*., 2009). Some of the most abundant saprophytes on the leaves of barley, and those responsible for the most frequent penetration attempts, are species of the genus *Cladosporium* (Smedegaard-Petersen and Tolstrup, 1985).

The aim of this project was to identify how fungicides increase the resource use efficiency and yield of spring barley so that fungicide strategies can be tailored more effectively to account for the disease resistance of the variety and the potential physiological response of the crop. Specifically, three broad research questions were addressed which have scientific and commercial relevance:

- What duration of protection of canopy light interception is required post-anthesis to maximise yield?
- How do fungicides increase yield where visible disease severity is low or absent?
- How should fungicides be timed in low disease risk situations?

The specific objectives were to:

1. Quantify the duration of green canopy protection required post-flowering to maximise yield.
2. Measure the importance of disease control during grain filling and the period around flowering when potential grain size is being determined.
3. Identify the mechanisms by which fungicides increase grain numbers and hence improve sink capacity in the absence of visible disease.
4. Test responses of sink components to fungicide treatment at key timings on a range of varieties with high ratings for disease resistance in the Recommended List.
5. Test new understanding across contrasting varieties and environments to develop commercial 'best practice' for disease management and improved yield of high quality grain (industry partner contribution).
6. Calculate the impact of improved disease control on resource use efficiency and greenhouse gas costs of production.

The project was structured such that the detailed physiological measurements needed to answer questions relating to the required duration of post-anthesis protection and the mechanisms of fungicide action were conducted at two research sites, SRUC Edinburgh and ADAS Rosemaund. Industry partners conducted experiments over a range of sites and varieties to test the yield response of spring barley to the same fungicide products under contrasting disease pressure.

Footnote: The above objectives are the same as those stated in the original project proposal, but objectives 1 – 3 in the proposal have been renumbered and reordered in this report to aid presentation of the research findings.

3. Materials & methods

3.1. Duration of canopy protection required post-anthesis (objective 1).

3.1.1. Experimental approach

It would be almost impossible to start and stop a disease epidemic in the field with sufficient precision to determine the duration of canopy protection required. For this reason shading was used to mimic the effects of severe disease on canopy light interception. Commencing at 50% ear emergence (Zadoks growth stage (GS) 55; Tottman, 1987) shade netting was erected at weekly intervals over plots of spring barley and left in place until harvest. Disease was prevented by using a variety (cv. Westminster) with good resistance to foliar disease and the application of a robust fungicide programme. The theoretical relationship between the onset of shading and grain yield is shown in Fig. 1. The netting reduced incident PAR (photosynthetically active radiation) at the top of the canopy by 64% at ADAS and 69% at SRUC. The small differences in extent of shading between sites may be the result of differences in tension applied to the netting. The duration of

protection of canopy light interception required to maximise yield was estimated as the period between GS55 and the time at which shading had no significant effect on yield (Fig. 1).

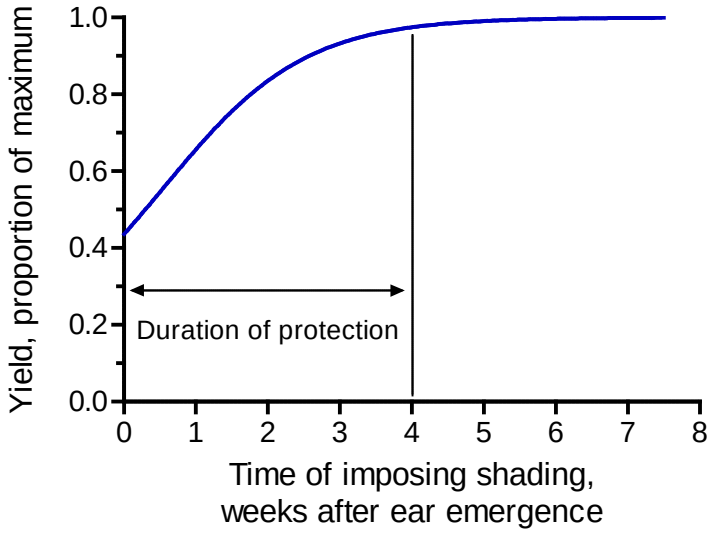


Fig. 1. Theoretical relationship between time of erecting shade netting and grain yield. Shades were erected at weekly intervals commencing at ear emergence and once erected were left in place until harvest.

3.1.2. Sites and experimental design

Experiments were conducted at ADAS in 2009 and SRUC in 2010 and 2011. Fields occupied a rotational position that was representative of barley production in the region. Full site and husbandry details are given in Appendix 1 and only a summary is outlined here. Plots (10 x 2 m) of spring barley (*Hordeum vulgare* cv. Westminster) were drilled at a viable seed rate of 360 seeds m^{-2}. Fertilizer N was applied at rates recommended for high yielding crops based on previous cropping and/or soil analysis (Sinclair *et al.,* 2009; Defra 2010). Fertilizer P, K and S were applied according to soil mineral analysis and anticipated crop demand. Micronutrients, herbicides and insecticides were applied to all plots as per standard farm practice with the aim of avoiding nutrient deficiency and providing robust weed and pest control. Disease was controlled using prophylactic applications of prothioconazole (Proline @ 0.4 l ha^{-1}) plus pyraclostrobin (Comet 200 @ 0.63 l ha^{-1}) at GS15–30 and GS39–45.

The experimental design was a complete randomised block with three replicate plots per shading treatment. Plots were drilled as close as possible to an east-west direction. Within a block, three discard plots were drilled between each experimental plot and a discard area exceeding 10 m in length sown between adjacent blocks. Discards were to prevent shadows being cast from shaded plots onto non-shaded experimental plots both within and between blocks. Shortly before ear emergence, fence posts 1.5 m in height above ground level were erected around the plots. From GS–55 onwards shade nets (Haygrove Ltd, Ledbury, UK) were erected at weekly intervals over the

designated experimental plot and its adjacent discard plots. The netting was secured to a support structure of fence wire running between the posts. At the ends of the plots (E and W) the netting was secured below canopy height to prevent direct light penetrating under the shading when the solar zenith angle (from the vertical) was large. Along the N and S edges, the netting was secured 1.2 m above ground level to provide adequate ventilation under the shade, whilst preventing ambient light reaching the experimental plot. The netting was constructed of an open mesh of black polyethylene that allowed rainfall to penetrate whilst restricting transmission of PAR.

Fig. 2. Post-anthesis shading at SRUC 2010

3.1.3. Measurements

At weekly intervals, 20 shoots were sampled at random from along the length of each experimental plot prior to the shade netting being erected. Shoots were separated into three fractions; leaf laminae, stem plus leaf sheaths and ears and each fraction dried in a fan assisted oven at 80^{o}C for 48h for dry weight determination.

Absolute leaf area, % green leaf area (GLA) and disease severity were determined every two weeks commencing at GS55 on a further ten shoots sampled at random per plot. Shoots were cut at ground level, sealed in a polythene bag to prevent moisture loss and transferred to the laboratory for assessment. Samples were assessed immediately or stored in their plastic bags in the dark at 4^{o}C for up to 48 h. Leaf laminae and stem were divided into fractions that corresponded to individual leaf layers, stem sections between successive leaves (incorporating stem and leaf sheath), peduncle and ear. Disease severity was assessed on the upper side of each fully emerged leaf by estimating visually the % area occupied by sporulating disease lesions, excluding the area of associated chlorosis. The latter was accounted for in a separate assessment of the %

GLA that considered both natural and disease-induced chlorosis and necrosis. Disease and % GLA were assessed in the same way on each section of stem and on the peduncle and ear. Colour reference charts were used to standardise assessment of GLA between SRUC and ADAS. After assessment of disease and % GLA, the absolute projected area of each fraction was determined using a leaf area meter (Li-Cor Biosciences, Lincoln, USA).

Within a day or two of sampling for disease assessment, the % of incident PAR intercepted by the canopy was determined using a Sunscan Canopy Analysis System (Delta T Devices Ltd, Cambridge, UK). Simultaneous measurements were made of PAR above and below the canopy. Measurements below the canopy were made at approximately 45^{o} to the direction of the plant rows. In unshaded plots, 9 measurements were made at regular spacings along the length of the plot. In shaded plots 45, measurements were made of PAR interception; 5 successive measurements at each of the 9 locations per plot. Here, incident PAR above the canopy was recorded under the shade. Movement of the shade netting in the wind can lead to spatial and temporal variability in incident PAR above the canopy. The repeated measures of PAR interception at each location in the plot were to minimise errors resulting from this. The reduction in PAR incident on the canopy as a result of the shading was quantified by measuring incident PAR above the canopy simultaneously in shaded and unshaded plots.

Between GS83 and final harvest, the number of ear bearing shoots along a 0.5 m length of row were counted at eight random locations per plot. Lodging was recorded if shoots were leaning greater than 45^{o} from the vertical, and the extent of lodging estimated as the % of the plot area affected. The date of leaf and stem senescence was recorded when less than 5% of shoots had green area remaining on leaf laminae or stem, respectively.

Temperature, relative humidity and rainfall were logged continuously under the shade and in an adjacent unshaded area. Soil cores to 90 cm were taken every two weeks, or prior to harvest depending on the site-year, from shaded and unshaded discard plots for determination of gravimetric soil moisture content. Cores were divided into 30 cm depth intervals, stones removed by hand and gravimetric water content determined after drying the soil at 100^{o}C for 48h.

At harvest maturity, shades and posts were removed and plots harvested by small plot combine. A sample of grain was taken for determination of mean grain weight and moisture content.

3.2. Importance of disease control during grain filling (objective 2)

3.2.1. Experimental approach

Three disease susceptible varieties were grown and different fungicide timings used to manipulate the duration and timing of post-anthesis disease epidemics. The rationale was that an early

fungicide application at the start of stem extension (T1, GS30–31) would be expected to provide relatively little protection during the post-anthesis period. A T1 application followed by a second application (T2), 2, 4 or 6 weeks later (broadly equivalent to GS37, 49–55 and 71, respectively) would give protection of the canopy lasting progressively later into the grain filling period. Assessments of the effects of the different fungicide timings on disease, % green leaf area and healthy area light interception, and their relationship to grain yield, could then be interpreted in terms of the duration of canopy protection required (identified in objective 1).

3.2.2. Sites and experimental design

Experiments were conducted at ADAS in 2011 and SRUC in 2011 and 2012. Plots (10 x 2 m) of spring barley were drilled at a viable seed rate of 350–360 seed m^{-2}. At ADAS, the experiment was laid out in a randomised block design with 4 replicate blocks. Treatments consisted of 3 varieties (Optic, Forensic and Waggon) and 5 fungicide timings (untreated, T1 only, T1 plus T2 at 2, 4 or 6 weeks after T1). At SRUC a split-plot design was used with 4 replicate blocks. Varieties were randomised within main plots and fungicide treatments in sub-plots. Fertiliser N was applied at rates recommended for high yielding crops based on previous cropping and/or soil analysis (Sinclair, *et al*., 2009; Defra, 2010). Fertiliser P, K and S were applied according to soil mineral analysis and anticipated crop demand. Micronutrients, herbicides and insecticides were applied to all plots as per standard farm practice with the aim of avoiding nutrient deficiency and providing robust weed and pest control. Fungicide treatments were prothioconazole (Proline @ 0.4 l ha^{-1}) plus pyraclostrobin (Comet 200 @ 0.63 l ha^{-1}) at T1 and T1 plus T2, with T1 being applied at GS30–31.

3.2.3. Measurements

Absolute leaf area, % green leaf area (GLA) and disease severity were determined every two weeks commencing at GS55 on ten shoots sampled at random per plot as described in section 3.1.3. PAR interception by the canopy was determined within a day or two of disease sampling using a Sunscan Canopy Analysis System (Delta T Devices Ltd, Cambridge, UK). Simultaneous measurements were made of PAR above and below the canopy at nine locations along the length of the plot. Ear numbers, the date of leaf and stem senescence, grain yield and mean grain weight were determined as described above (section 3.1.3).

3.3. Mechanisms by which fungicides increase grain numbers and yield in the absence of visible disease (objective 3)

3.3.1. Experimental approach

Two complimentary experimental approaches were used to investigate the mechanisms of yield responses to fungicide where visible foliar disease was negligible or absent. The first involved

shading crops during the stem extension period to establish the relationship between pre-anthesis PAR interception, grain number formation and yield for both fungicide-treated and untreated crops. Pre-anthesis disease was kept to a minimum in each set of treatments by using the variety Westminster which has good resistance to the main disease threats, mildew, rhynchosporium and ramularia (HGCA Recommended List 2013). The response of fungicide-treated crops was compared with that of untreated crops to test the hypothesis that a yield response to fungicide in the absence of visible disease is associated with an increase in pre-anthesis PAR interception by healthy tissue. Samples of leaves were taken for determination of endophytic (symptomless) *Rhynchosporium commune* and *Ramularia collo-cygni* infection, using PCR assays. This was to test the hypothesis that an increase in grain number formation and yield in the absence of visible disease could result from control of asymptomatic infection. If neither of these hypotheses was proven, the alternative explanation that fungicide treatment increases yield through effects on RUE and biomass partitioning via control of epiphytic saprophytes or direct effects on plant metabolism would be invoked.

The second approach involved a comparison of the effects of different fungicide chemistries on PAR interception, asymptomatic pathogen infection and yield formation in the absence of visible disease. The fungicide active ingredients compared were prothioconazole, pyraclostrobin and chlorothalonil. These are representative examples of the triazole, strobilurin and chlorophenyl groups of fungicides respectively. Triazoles have been reported to have anti-gibberellin activity, whilst the strobilurins have been reported to influence cytokinin and ethylene metabolism. Chlorothalonil, on the other hand, has not been linked to effects on plant metabolism. If control of saprophytes and asymptomatic pathogen infection by the different chemistries was found to be comparable, yield enhancement in the absence of visible disease by triazoles and/or strobilurins, but not chlorothalonil, would suggest that the mechanism involves a direct effect on plant metabolism.

3.3.2. Sites and experimental design

Pre-anthesis shading experiments were conducted at SRUC in 2009 and 2010 and at ADAS in 2009 and 2011. At each site, the experiment was a factorial combination of two levels of shading (shaded and non-shaded) and two levels of fungicide (treated and untreated). The experiment was laid out in a split-plot design with replicate 4 blocks. The shade treatment was randomised within main plots and the fungicide treatment within sub-plots. In order to minimise the risk of a disease epidemic developing without fungicide treatment, experiments were conducted on the spring barley variety Westminster. Plots (10 x 2 m) were drilled in an E-W direction at a viable seed rate of 360 seed m^{-2}. Three discard plots were sown between each pair of experimental plots allowing shades to be extended beyond the experimental plots. This was to prevent exposure of the experimental plots to light penetrating under the edges of the shades and to avoid shadows being cast from the

shades onto neighbouring non-shaded plots. Fertiliser, herbicide and insecticide applications are detailed in Appendix 1. Fungicide-treated plots were sprayed with prothioconazole (Proline @ 0.4 l ha^{-1}) plus pyraclostrobin (Comet 200 @ 0.63 l ha^{-1}) at GS15–30 (T1) and GS45–49. In 2009 all plots (fungicide-treated and untreated) received an overspray of chlorothalonil (Bravo 500 @ 1.0 l ha^{-1}) at GS45–49 to prevent visible post-anthesis ramularia infection. In 2010 and 2011, the chlorothalonil overspray was omitted. Shades were erected at GS30 after the first fungicide treatment had been applied as described above (section 3.1.2) and removed at GS55–5.

The effects of fungicide chemistry on yield were investigated in experiments conducted at ADAS in 2009, 2011 and 2012 and at SRUC in 2010 and 2012. In 2012, SRUC experiments were conducted at two sites; one at Boghall farm, Edinburgh, the other at MacRobert Farm, Craibstone, Aberdeen. In 2009, 2010 and 2011, experiments were conducted on a disease resistant (Westminster) and a susceptible (Optic) variety. In 2012, the treatments were modified and only the resistant variety Westminster was used. In each case the experimental design was a randomised block with 4 replicates. Fungicide treatments were chlorothalonil (Bravo 500 @ 1.0 l ha^{-1}), prothioconazole (Proline @ 0.4 l ha^{-1}), pyraclostrobin (Comet 200 @ 0.63 l ha^{-1}), prothioconazole plus pyraclostrobin (at the same rates as when applied singularly). Untreated plots served as controls. All fungicides were applied at both T1 (GS30–31) and T2 (GS45–49). General husbandry details are given in Appendix 1.

3.3.3. Measurements

In pre-anthesis shading experiments, disease and % GLA were assessed by leaf layer on 10 plants (GS15 and 31) or shoots (GS39, 59 and 75) sampled at random from along the length of the plot. At GS15 and 31 main shoots were identified and the leaf laminae separated from the leaf sheaths. Disease severity and % GLA were assessed visually on each main shoot leaf layer and the leaf sheath fraction as described above (section 3.1.3). An average disease severity and % GLA across all tiller leaf layers and the tiller leaf sheaths were also recorded. At GS39 and beyond, shoots were divided into individual leaf layers, stem sections between successive leaf layers, and (after its emergence) the ear, for assessment of % disease severity and % GLA (section 3.1.3). After assessment, the absolute projected area of the same tissue fractions was determined using a leaf area meter (Li-Cor Biosciences, Lincoln, USA).

PAR interception by the crop canopy was measured within two days of sampling for disease assessment using a Sunscan Canopy Analysis System (Delta T Devices Ltd, Cambridge, UK) as described in section 3.1.3. At GS15–31, GS39, GS59 and GS75 (depending on the year) a further 20 plants (or main shoots at GS59 and 75) were sampled along the length of each plot. Laminae from the uppermost four main shoot leaves were excised, bulked into one sample per plot, frozen and stored at -20^{o}C to await PCR analysis of rhynchosporium and ramularia. Leaf samples from

ADAS were chilled to 4^{o}C immediately after sampling, dispatched on ice to SRUC and frozen within 24 h of sampling. For extraction of total genomic DNA, leaf tissue was ground in liquid N_2 and DNA extracted using the Nucleon® Phytopure Plant DNA extraction kit (GE Healthcare UK Ltd, Buckinghamshire, UK), according to the manufacturer's instructions. Extracted DNA was quantified spectrophotometrically in a BioPhotometer (Eppendorf AG, Hamburg, Germany). *R. commune* and *R. collo-cygni* DNA in the extracts was quantified by real-time PCR following the methods of Fountaine *et al*. (2007) and Taylor *et al*. (2010). All PCR analysis was conducted at SRUC.

Four separate 0.3 m row lengths were marked at random locations along the third row in from each side of the plot (eight row lengths in total). Viable shoot numbers were counted weekly (2009) or every two weeks (2010 and 2011) in the marked rows from early tillering until mid grain filling. Shoots that had begun to die were not counted. A dying shoot was defined as one that had begun to senesce and turn yellow, commencing with the youngest (uppermost) leaf. Final ear numbers, lodging, the date of canopy senescence, grain yield and mean grain weight were measured as described above (section 3.1.3).

Air temperature, relative humidity and rainfall were logged continuously under the shade and in an adjacent non-shaded area. Soil cores to 90 cm were taken every two weeks during the period of shading from shaded and non-shaded discard plots for determination of gravimetric soil moisture content. Cores were divided into 30 cm depth intervals, stones removed by hand and gravimetric water content determined after drying the soil at 100^{o}C for 48h.

In 2009 and 2010, experiments on the effects of contrasting fungicide chemistries, disease and % GLA were assessed at GS31, 39, 59 and 75 as described above. In 2011 and 2012, these assessments were accompanied by measurements of canopy PAR interception and absolute leaf area enabling a more detailed analysis of light interception by healthy tissue to be made. In addition, samples were taken of the top four main shoot leaves to quantify rhynchosporium and ramularia DNA by real-time PCR as described above. Measurements of final ear number, date of canopy senescence, lodging (if any), yield and grain quality were made as outlined previously.

At ADAS 2012, samples of the leaf 2 (leaf below the flag leaf) were taken from plots of Westminster treated with the different fungicide products and examined microscopically to quantify leaf surface fungi. Five leaves were sampled at random from each plot at GS59, placed in a polythene bag and stored on ice in a cool box for transport to the laboratory. A 3 cm long mid-section of leaf was excised, boiled for 1 min in 0.025% Trypan blue, left to stain overnight, and then destained in chloral hydrate for examination under a light microscope. The number of fungal hyphae and spores on the upper surface of the lamina and along the main midrib were counted in five random fields of view per leaf and numbers expressed per unit area under observation.

At SRUC Edinburgh 2012, flag leaves of Westminster were sampled from the same fungicide treatments for determination of *Cladosporium* sp. DNA on the leaf surface by quantitative real time PCR. The PCR assay was developed as part of an HGCA summer bursary to Judit Bliss.
Ten flag leaves were sampled at random from plots at GS39 and GS59 and stored at -20^{o}C until analysis. A subsample of five flag leaves were placed into scintillation vials and sonicated for 7 min in 10 ml 0.1 M potassium phosphate buffer (pH 7.0) to remove epiphytic microorganisms from the leaf surface. DNA was extracted from the washings and the amount of *Cladosporium sp* DNA quantified by qPCR using genus specific primers following the methods described by Bliss *et al.* (2012).

3.4. Effects of fungicide timing on yield and sink components in disease resistant varieties (objective 4)

3.4.1. Experimental approach

To extend understanding of the effects of fungicide on yield and in particular grain number formation in the absence of visible disease, experiments were conducted to investigate whether a particular fungicide timing is required to elicit the response. The range of resistant varieties used was also extended, compared to previous experiments, to test the whether the observed responses to fungicide have general applicability across varieties.

3.4.2. Sites and experimental design

Experiments were conducted at ADAS and SRUC in 2011 and 2012. At SRUC 2012, experiments were located at two sites; Boghall farm Edinburgh and MacRobert Farm, Craibstone, Aberdeen. Experiments consisted of three spring barley varieties (Westminster, Quench and Garner) in factorial combination with four fungicide timings (untreated, T1, T2, T1 + T2). At each timing the fungicide treatment was prothioconazole (Proline @ 0.4 l ha^{-1}) plus pyraclostrobin (Comet 200 @ 0.63 l ha^{-1}). The timings were T1, GS30–31 and T2, GS45–49. At ADAS in 2011 the experiment was laid out as a randomised block with 4 replicates, whilst in the other site-years the experiment was a split-plot design with 4 replicates; varieties were randomised within main plots and fungicide treatments within sub-plots. Plots (10 x 2 m) were drilled at a seed rate of 350–360 seeds m^{-2}. Full site and husbandry details are given in Appendix 1.

3.4.3. Measurements

In 2011, disease severity, % GLA and canopy PAR interception were assessed at two weekly intervals commencing GS31 (corresponding to measurements at GS31, and approximately, GS39, 59 and 75) as described previously. No measurements of absolute leaf area were made. In 2012,

disease, % GLA, PAR interception and absolute leaf area were measured at the same target growth stages as those in 2011. An additional assessment of %GLA and absolute area were made at GS59+4 weeks.

At GS31, 39, 59 and GS59+2 weeks 10 plants (10 main shoots at GS59 and GS59+2 weeks) were sampled at random along the length of the plot. The samples were processed immediately upon return to the laboratory. The top four fully expanded leaf laminae were excised from the main shoot, bulked to give one pooled sample for all ten plants or shoots per plot and frozen at -20^{o}C to await analysis of *R. commune and R. collo-cygni* DNA by real time quantitative PCR as described above. Samples from ADAS and SRUC Aberdeen were refrigerated and transported at 4^{o}C to SRUC Edinburgh. They were then frozen and stored prior to analysis.

Final ear numbers per m^2, the date of canopy senescence, the extent of lodging, yield and mean grain weight were determined at all sites and years as outlined previously. Immediately prior to harvest in 2012, grab samples were taken at SRUC Edinburgh and Aberdeen for determination of harvest index. Grab samples consisting of approximately 10–15 shoots were taken at 5 random locations along the plot. Shoots were cut at ground level and the number of ear bearing and non-ear bearing shoots counted before separating into ears and straw and drying at 80^{o}C in a fan assisted oven for 48h. The ears and straw were weighed, and the grains separated using a laboratory thresher (Wintersteiger LD180, Austria). The threshed grains were weighed and the chaff collected and added to the straw fraction. The weight of chaff was calculated as the difference in ear and grain weights. In 2012, the N concentration of grain (and straw at SRUC) was determined following Kjeldahl digestion of milled samples. The tissue was taken from grab samples at SRUC and samples from the combine harvested grain at ADAS.

3.5. Calculations and statistical analysis (objectives 1-4)

Unless otherwise stated, grain yields and mean grain weights (MGW) are expressed on the basis of 85% dry matter. Grain number per m^{-2} was calculated as grain yield/MGW. Ear counts along defined lengths of plant rows were converted to ear number m^{-2} by dividing by the row width.

PAR interception by healthy tissue was estimated using methods adapted from Bingham *et al.* (2012a). A canopy area index (CAI, total projected area per unit ground area) was calculated from the PAR interception measurements using Beer's law analogy assuming a light extinction coefficient (*k*) of 0.6 (equation 1):

$$CAI = [\ln (I_t/I]/k \qquad 1$$

where I is the incident PAR and I_t is the PAR transmitted to the base of the canopy.

From GS39 onwards, measurements of absolute leaf and stem plus leaf sheath area were used to calculate the proportional distribution of projected area in five zones representing the top five leaf layers. The planar area for a particular leaf layer was given by the sum of the lamina area in that layer and the stem section between the leaf and the one above it. In the case of the flag leaf layer (leaf layer 1), the area consisted of the leaf lamina plus the peduncle. The stem below leaf five plus any remaining senesced non-culm leaves were included in the leaf 5 layer. After ear emergence the ear comprised an additional layer. The projected area in each layer was expressed as a fraction of the sum of all layers. The fractional distribution of projected area from the measured samples was used to estimate the *CAI* in each layer as (equation 2):

$$CAI_h = CAI \times fLA_h \qquad 2$$

where CAI_h is the *CAI* of layer *h* and fLA_h is the projected area of layer *h* expressed as a fraction of the total area.

PAR intercepted daily by each layer was then calculated as:

$$I_h = I_{oh} \times [1 - \exp(-k \times CAI_h)] \qquad 3$$

where I_h is the PAR intercepted daily by layer *h*, I_{oh} is the daily PAR incident on layer h, and *k* is the assumed extinction coefficient of 0.6. I_{oh} was calculated as the difference between the daily amount of PAR incident on the top of the canopy (I_o) and the sum of that intercepted by all layers above layer *h*.

The PAR intercepted by healthy (green) tissue in a given layer h was then given as:

$$HA_{inth} = I_h \times [HAI_h/CAI_h] \qquad 4$$

where HA_{inth} is the healthy area PAR interception by layer *h* and HAI_h/CAI_h is the fraction of the canopy area index in layer *h* that is healthy (green). The latter was calculated from a weighted average of the measured % GLA values of leaf lamina and stem plus leaf sheath for the layer in question.

HA_{int} for the canopy as a whole was calculated as the sum for the individual leaf and ear layers and expressed as the fraction (F_{PAR}) of the incident PAR for the day I_o:

$$F_{PAR} = HA_{int}/I_o \qquad 5$$

Equations 1–5 were used to calculate GA_{int} and F_{PAR} interception for growth stages at which PAR interception and disease assessments were stratified by leaf layer (i.e. GS39 onwards). At GS31 F_{PAR} was calculated as:

$$F_{PAR} = (1- [I_t/I]) \times fGLA_w \quad 6$$

where (1- $[I_t/I]$) is the measured value of fractional PAR interception by the canopy at GS31 and $fGLA_w$ is the weighted average of visual GLA assessments across the different tissues (main shoot lamina, leaf sheaths and tillers) expressed as a fraction of the total tissue area rather than a %.

To estimate HA_{int} over a given interval between growth stages, the value of F_{PAR} for each of the bounding growth stages was averaged and multiplied by the sum of the daily incident PAR for the interval. The above method of estimating PAR interception by healthy tissue takes into account the distribution of disease within the canopy. It also assumes that PAR incident on necrotic and chlorotic tissue is intercepted and not reflected or transmitted to neighbouring green healthy tissue.

Statistical analysis was by ANOVA for completely randomised or split-plot designs using Genstat 14th Edition (VSN International Ltd., Hemel Hemstead, UK). Residuals were checked for homogeneity of variance and normality of distribution and transformed where necessary. Percentage values for individual diseases and disorders were arcsine transformed prior to analysis. Back-transformed mean values are presented. Where a cross site-season analysis has been carried out, site-season effects were analysed as random effects with fungicide treatments and varieties as fixed effects.

3.6. Test understanding across contrasting varieties and sites (objective 5). Industry partner contribution

3.6.1. Experimental approach.

Experiments to investigate the mechanisms of fungicide effects on yield were necessarily confined to just two core research sites and a limited number of varieties to enable the detailed physiological measurements to be made. In order to establish the general validity of the findings for spring barley production across the UK, it was important to test responses to fungicide on a larger number of varieties at a wider range of locations differing in disease pressure. Spring barley experiments were contributed by industry partners from 2009 – 2011, providing data from a total of 30 variety/site experiments (Appendix 2). The partners were asked to apply the same fungicide treatments as those used at the ADAS and SRUC core research sites. Proline (0.40 l ha^{-1}), Comet 200 (0.63 l ha^{-1}) and Proline plus Comet (0.40 and 0.63 l ha^{-1}, respectively) were applied at each site at T1 (GS25–31) and at T2 (GS39–59); untreated plots served as controls. Experiments were

laid out in a randomised block design. Other non-fungicide treatments (herbicide, insecticide, fertiliser) were applied to all plots, as per the standard farm practice of each partner. No growth regulators were applied.

3.6.2. Assessments and yield

Disease severity and % green leaf area were assessed at growth stages 30–31, 39, 59–69 and 75–80. Disease was assessed as the % area covered by lesions for each individual leaf layer on 10 randomly selected plants per plot. Records were made of missing leaves and leaves where no disease was present. Ears m^{-2} were determined between mid-grain fill and harvest by counting the number ears along a 0.5 m length in six randomly selected areas per plot, avoiding the outer two rows of plots and any atypical areas. Plots were combine-harvested and grain yield, grain moisture content, mean grain weight (MGW) and specific weight determined. All data records were sent to ADAS for analysis. The number of grains m^{-2} was calculated as grain yield/MGW.

3.6.3. Soil Moisture Deficit analysis

Predictions of soil moisture deficit (SMD) per day were made for each site and year using the model 'Irriguide', v4.3 (Silgram *et al*., 2007). The input data included: dates of drilling, T1 application, T2 application and harvest; soil texture; previous crop; grid reference. The assumptions used to obtain SMD predictions were a default rooting depth of 90 cm, crop cover 55% at GS24, 75% at GS30 and 90% at GS31. Drought stress was defined as when the SMD was greater than the easily available water capacity (water held at less than 2 bar tension) in a 90cm soil profile (expected maximum effective rooting depth).

3.7. Impact of improved disease control on resource use efficiency and greenhouse gas costs of production (objective 6)

The effects of fungicide chemistry and timing on the greenhouse gas (GHG) costs of production and N use efficiency were calculated using data from experiments conducted in 2012 as data were only available for grain and straw N in 2012. GHG emissions in carbon dioxide equivalent (CO_2e) associated with the production of a tonne of spring barley were calculated using a PAS2050 compatible approach (BSi 2011) as described by Berry et al. (2008, 2010) and Sylvester-Bradley *et al.* (2012). Calculations were made for each of the three sites using average treatment yields. Emissions were calculated to the farm gate, with yields adjusted to 85%DM. Elements in the calculations included; emissions associated with the manufacture of fertilisers (NPK and lime) and pesticides; all diesel use for cultivation, spraying, fertiliser application and harvest (using default figures for diesel use); nitrous oxide emissions from nitrogen fertiliser applications and from residue

management. The rates of application/use were multiplied by standard emissions factors to calculate the emissions per hectare of production. This figure was then divided by the yield per hectare to give an estimate of emissions per tonne of grain produced. Within sites, cultivations, rates, type of fertiliser and pesticides other than fungicides were identical for each experimental treatment. Treatments differed in yield, the dose of active ingredient applied and the number of spray passes required.

Effects of fungicides on grain N offtake were calculated from measured values of grain yield and grain N concentration. As a pre-sowing analysis of soil, mineral N was not available for all sites (sampling was delayed until after sowing and fertilizer application at SRUC Edinburgh), NUE was calculated as the grain yield per kg fertiliser N applied. Additional measurements of straw biomass and N concentration at SRUC sites enabled a more complete analysis of components of NUE to be made at these sites, including total N offtake, dry matter harvest index (HI), nitrogen harvest index (NHI), N utilisation efficiency for grain and total biomass, as described by Bingham *et al*., (2012b). As no estimate of soil N supply was available from these experiments, the ratio of total N offtake to fertiliser N applied was calculated as an index of the N-fertiliser uptake efficiency.

4. Results & Discussion

4.1. Duration of canopy protection required and importance of disease control around and after flowering (objectives 1 & 2)

4.1.1. Response to post-anthesis shading

The effects of post-anthesis shading on yield conformed to the theoretical relationship shown in Figure 1. Thus, when plots were shaded from GS55 through to harvest, there was a large and significant ($P<0.001$) reduction in yield compared to non-shaded plots (Fig. 3). The reduction ranged from 19% to 77% depending on the site-year. As the shading was imposed progressively later during the grain filling period, its effects on yield diminished. The overall pattern of response was similar in each of the site-years although the magnitude of the effect on yield differed. The time at which there was no further significant reduction in yield by shading was around 3 weeks after GS55 at SRUC in 2010, 4 weeks at ADAS 2009 and 5 weeks at SRUC 2011 (Fig. 3). At these times there was still an appreciable amount of green leaf area on the crop. Thus, the average % GLA across the top 4 leaves was in the range 25–50%. Stem and ear % green area was greater (data not shown). These data suggest that the PAR interception by the canopy must be protected for a period of 3–5 weeks after 50% ear emergence (GS55) in order to maximise yield.

The effects of shading on yield cannot be ascribed to changes in meteorological conditions other than the reduction in radiation. Shading had negligible effects on mean air temperature and relative

humidity (RH) above the canopy; on average the air temperature was just 0.1 °C and the RH 1.5–2.0% greater under the shade compared to open crops (Appendix 3). Measurement of rainfall under the shade was problematic. In one year the mean daily rainfall recorded was 17% lower under the shade, in the other it was 22% greater. Evidently, the shade netting allowed rainfall to penetrate, but it is likely that the netting reduced the uniformity of its distribution over the plot. It is to be expected that some pooling of water occurred on the netting before transmission through and thus the accuracy of the rainfall readings would be dependent on the siting of the rain gauge relative to the collection of water. It might also arise from the interception and collection of mist. This could account for why the mean daily rainfall recorded under the shade was greater in one instance than that over an unshaded crop. Importantly, however, shading had no significant effect on the gravimetric soil moisture content measured at any depth in the soil profile at the end of grain filling (Appendix 3) indicating that shading had little effect on soil water supply to the crop.

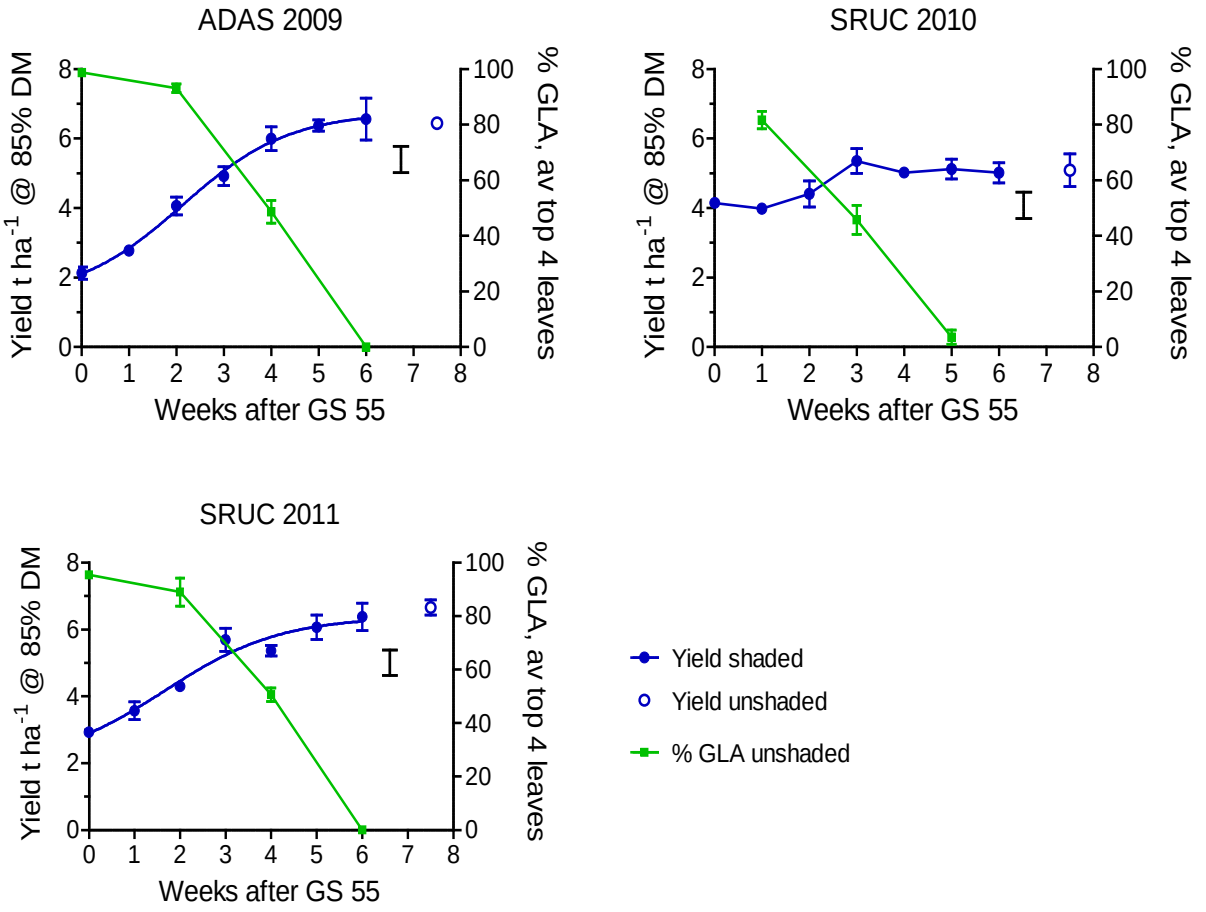


Fig. 3. Effects of the time of onset of shading on yield and the % green leaf area (GLA) averaged over the top 4 leaves for unshaded plots. Values are means ± SEM of 3 replicates. The stand-alone vertical bar represents the LSD (5% probability) for the effects of time of shading on yield following a one-way anova. A sigmoid function was fitted to yield data for ADAS 2009 and SRUC 2011. The fit was poor for SRUC 2010, and thus a non-fitted line is shown.

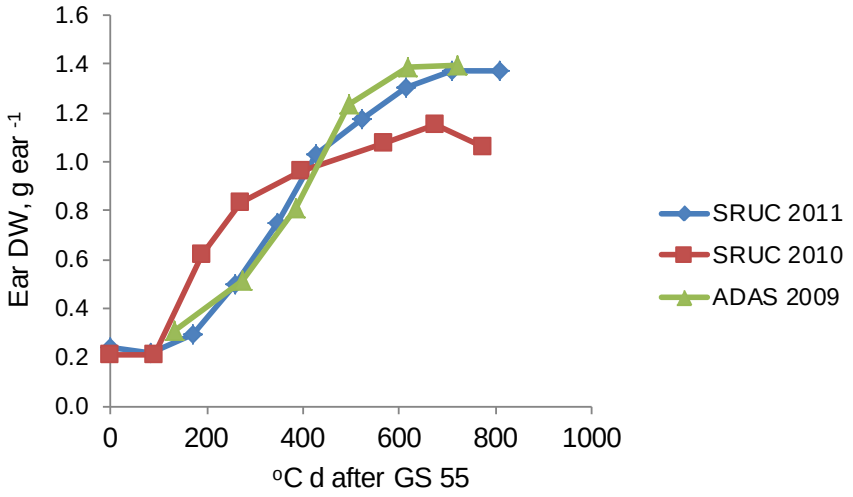


Fig. 4. Changes in ear dry weight of unshaded plots with accumulated thermal time (base temperature 0 °C) after GS55. Values are means of 20 ears per plot and three replicate plots. Error bars are omitted for clarity.

Table 1. Estimated duration of protection of canopy light interception required post-anthesis to maximise yield expressed in terms of calendar weeks or thermal time from GS55 or the fraction of grain filling completed (fraction of final grain weight attained).

	Duration of protection			Duration of grain fill	Unshaded final
Site	Weeks after GS 55	°C d after GS 55	Frac of grain fill	°C d after GS 55	yield, t ha^{-1} @ 85% DM
ADAS 2009	~ 4	495	0.85	721	6.4
SRUC 2010	~ 3	271	0.72	676	5.1
SRUC 2011	~ 5	427	0.75	711	6.7

The duration of grain filling was estimated from plots of ear dry weight against accumulated thermal time from GS55 (Fig. 4). Maximum ear dry weight was attained at a broadly comparable thermal time (approx 700 °Cd) in each of the site-years. These data allow the duration of canopy protection required to be expressed on the basis of both thermal time and the relative progress of grain filling (fraction of final grain weight attained) (Table 1). The results of the shading treatments suggest that canopy PAR interception must be protected until ~75% of grain filling has been completed (average across site-years = 77%). After that yield is relatively insensitive to major reductions in intercepted PAR. This equated to a thermal time period of 271–495 °Cd (degree days) from GS55 depending on the site-year.

Assimilates for grain filling may be derived from post-anthesis photosynthetic activity and from the mobilisation and re-translocation of pre-anthesis storage reserves (Grashoff and d'Antuono, 1997;

Bingham *et al*., 2007b). Using values for the fraction of grain filling that is insensitive to reductions in light interception and the final unshaded yield, it is possible to estimate the quantity of dry matter present in the grain that must have been derived from storage reserves. The quantity ranged from 0.82 to 1.43 t ha^{-1} which is within the range reported for stem water soluble carbohydrate reserves of barley crops (Bingham *et al*., 2007a; Bingham *et al*., 2012a). The values will be an overestimate of the dry matter supplied by storage reserves as the calculation assumes that photosynthesis was completely inhibited by shading, which was not the case. The variation in the duration of protection required between site-years, expressed in terms of the progress of grain filling, may in part arise from variation in the source-sink balance of the different crops. Considerable variation in source-sink balance has been found for winter barley crops across sites and years (Bingham *et al.,* 2007a). Crops with a large potential supply of assimilate relative to their grain sink capacity would be expected to require a shorter duration of post-anthesis canopy protection compared to crops whose source and sink are in closer balance. Differences in the rate of crop development between sites and years, in addition to the variation in source-sink balance, will contribute to the variation observed in duration of protection required when expressed on a calendar basis (Table 1).

4.1.2. Response to fungicide timing

Three varieties, with relatively low disease resistance ratings, were grown and fungicide timing used to vary the severity of disease around flowering and during grain filling. The severity of visible disease post-anthesis for untreated crops is shown in Fig. 5. The type and severity of disease or disorder differed between sites and years. At ADAS in 2011, the main diseases were powdery mildew (*Blumeria graminis* f.sp.*hordei*) and rhynchosporium leaf scald (*R. commune*); severities were low to moderate and each variety was infected to a similar extent. At SRUC in 2011, the severity of mildew and rhynchosporium was broadly comparable to that at ADAS, with the exception of Waggon, which had significantly less disease than Optic (mildew and rhynchosporium) and Forensic (rhynchosporium). However, the most significant loss of green area resulted from physiological brown spotting and here Forensic was the variety most severely affected. At SRUC in 2012 there was a major ramularia (*R. collo-cygni*) epidemic with average severities exceeding 30% of the leaf area for all varieties.

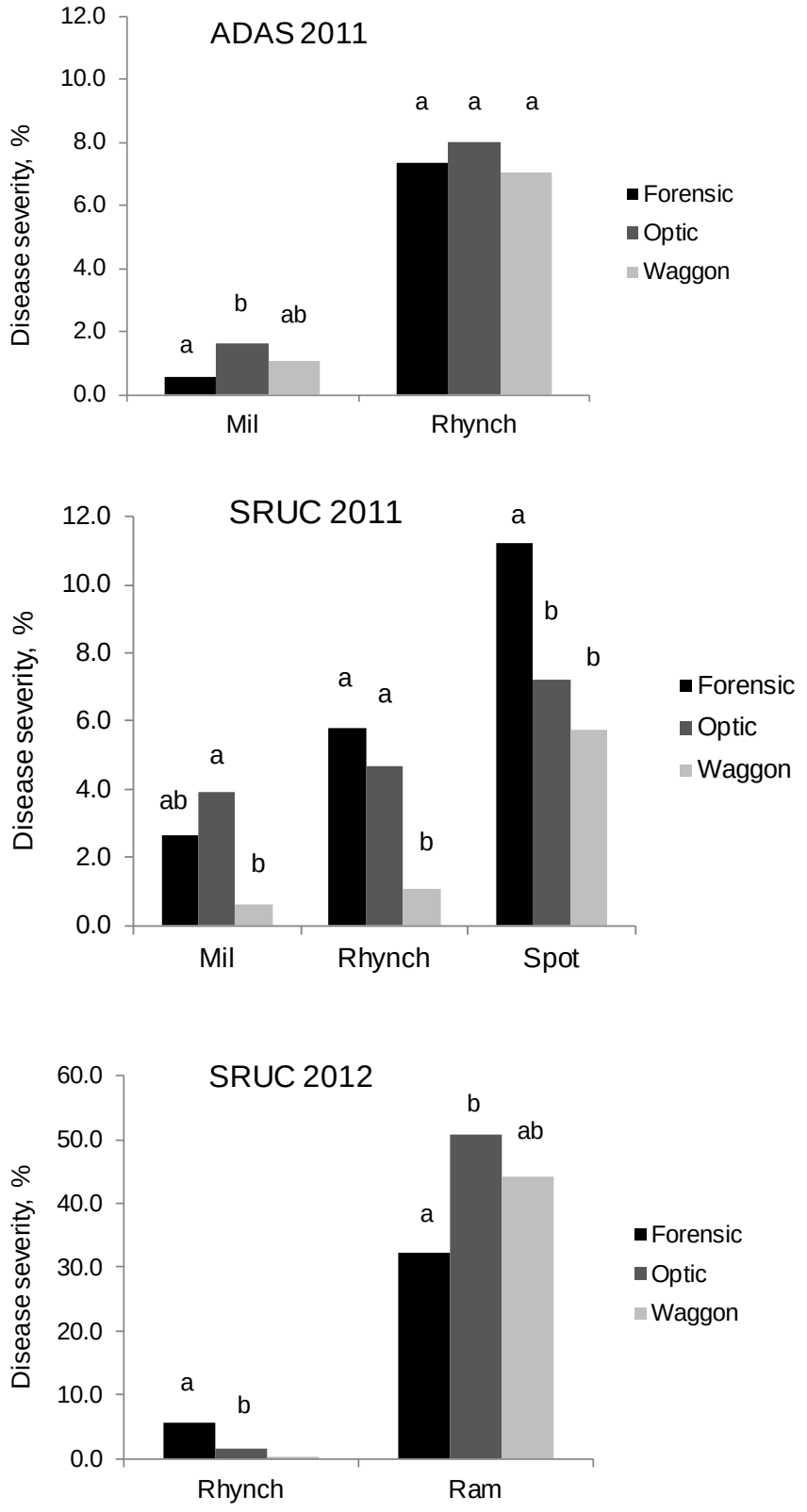


Fig. 5. Disease and disorder severity averaged over the top 3 leaves of untreated plots during grain filling (GS73–83). Data were arcsine transformed for ANOVA; values are back-transformed means. For a particular disease, varieties with a different letter are significantly different at P<0.05. Note the different scale used for SRUC 2012.

Table 2. Grain yield (t ha^{-1} @ 85% DM) in response to different fungicide timing combinations. For ADAS 2011 and SRUC 2012 there was no significant interaction between variety and fungicide timing and so only main effects of fungicide are shown.

	ADAS 2011		SRUC 2011			SRUC 2012	
Fungicide			Forensic	Optic	Waggon		
Untr	7.64		6.02	6.53	7.19	3.71	
T1 only	8.44		6.76	7.04	7.16	4.10	
T1 + T2 early	8.33		7.02	7.08	7.29	4.21	
T1 + T2 mid	8.56		7.01	7.07	7.24	4.38	
T1 + T2 late	8.51		7.03	7.23	7.20	4.40	
	p	*lsd*	*p*	*lsd*		*p*	*lsd*
V	<0.001		0.046	0.335		0.012	
F	<0.001	0.322	<0.001	0.235		<0.001	0.129
*V*F*	ns		0.041	0.458		ns	
same level of var				0.403			

There was a highly significant (P<0.001) yield response to fungicide treatment in each of the three–site-years (Table 2). At ADAS 2011 and SRUC 2012, there was no significant variety by fungicide interaction indicating that varieties responded to fungicide treatment in the same way. This is consistent with the relatively small, albeit sometimes statistically significant, differences between varieties in the severity of visible disease at these sites (Fig. 5). At SRUC in 2011, there was a significant interaction, with Waggon showing no overall response to fungicide in contrast to Optic and Forensic. Again this is consistent with the lower severities of mildew and Rhynchosporium on Wagon compared with the other varieties (Fig. 5).

With the exception of Waggon at SRUC 2011, a T1 application of fungicide gave a significant yield increase at all sites, ranging from 8% to 12%. An additional application at T2 gave a significant further increase in yield only at SRUC 2012, the site-year where there was a severe ramularia epidemic. Here, the greatest benefit of the T2 treatment (~0.3 t ha^{-1}) was associated with an application at the mid or late T2 timing (at the start of ear emergence (GS51–53) or at GS71 respectively). A comparable increase in yield (~0.3 t ha^{-1}) was observed following T2 applications on Forensic at SRUC in 2011, but this did not reach levels of statistical significance.

Estimates of PAR interception by healthy tissue can be used to integrate the effects of different foliar diseases on canopy green area and radiation capture during the post-anthesis period. When data from post-anthesis shading and fungicide timing treatments were pooled from different sites-years, there was a broad linear relationship between post-anthesis healthy area PAR interception and yield (Fig. 6). The exceptionally low yields at SRUC in 2012 were associated with low PAR interception resulting from low incident PAR during grain filling. It is evident that the effects of fungicide treatments on healthy area PAR interception and yield were relatively small compared to differences between sites-years and the effects of the shading treatments.

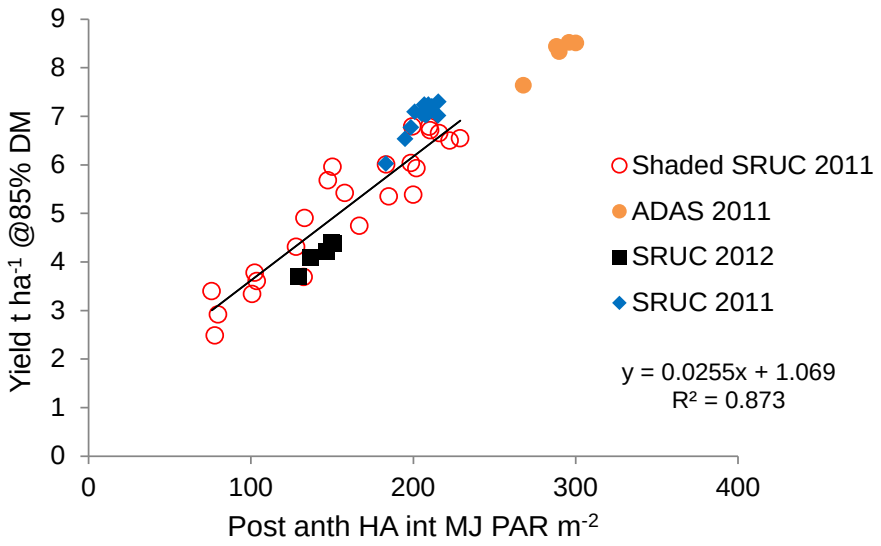


Fig. 6. Relationship between grain yield and post-anthesis PAR interception by healthy area. Data are from the post-anthesis shading experiment on variety Westminster (SRUC 2011) and fungicide timing experiments at ADAS and SRUC. At SRUC 2012 and ADAS 2011 values for fungicide timing treatments are means across varieties; at SRUC 2011 values are for individual varieties. Line fitted by linear regression to data from the shading experiment.

The results imply that high yields are dependent on achieving a large accumulated PAR interception by healthy tissue during the post anthesis period. However, whilst differences in yield between site-years were associated with differences in PAR interception, closer inspection of the results at particular site-years, indicates that the relationship between healthy area PAR interception and yield resulting from fungicide treatment was non-linear (Fig 7). Thus, at SRUC in 2011, yield increased with healthy area PAR interception up to a value of around 200 MJ m^{-2}, but ceased to increase above that. At ADAS in 2011, there was little further increase in yield above 290 MJ m^{-2}. These results suggest that in crops of differing yield potential, fungicide treatment can prolong canopy green area and PAR interception beyond that needed to maximise yield, presumably because the crop already has sufficient assimilation capacity and storage reserves available to meet the storage capacity of the grain. A comparable non-linear relationship has been reported between flag leaf lifespan and yield of wheat (Pepler *et al*., 2005). Increasing the lifespan with fungicide beyond 700^{o}C days failed to provide further increases yield. The authors attributed this to the onset of sink-limitation of grain filling.

The effectiveness of a given fungicide timing in protecting canopy PAR interception at different stages during grain filling is shown in Fig. 8. Results are expressed in terms of the fraction of incident PAR that is intercepted by healthy tissue. At or shortly after 50% ear emergence (GS55 to GS55+1 week) between 85 and 90% of the incident PAR was intercepted by healthy tissue in crops in each of the site seasons. In each case, those treated with fungicide at T1 intercepted

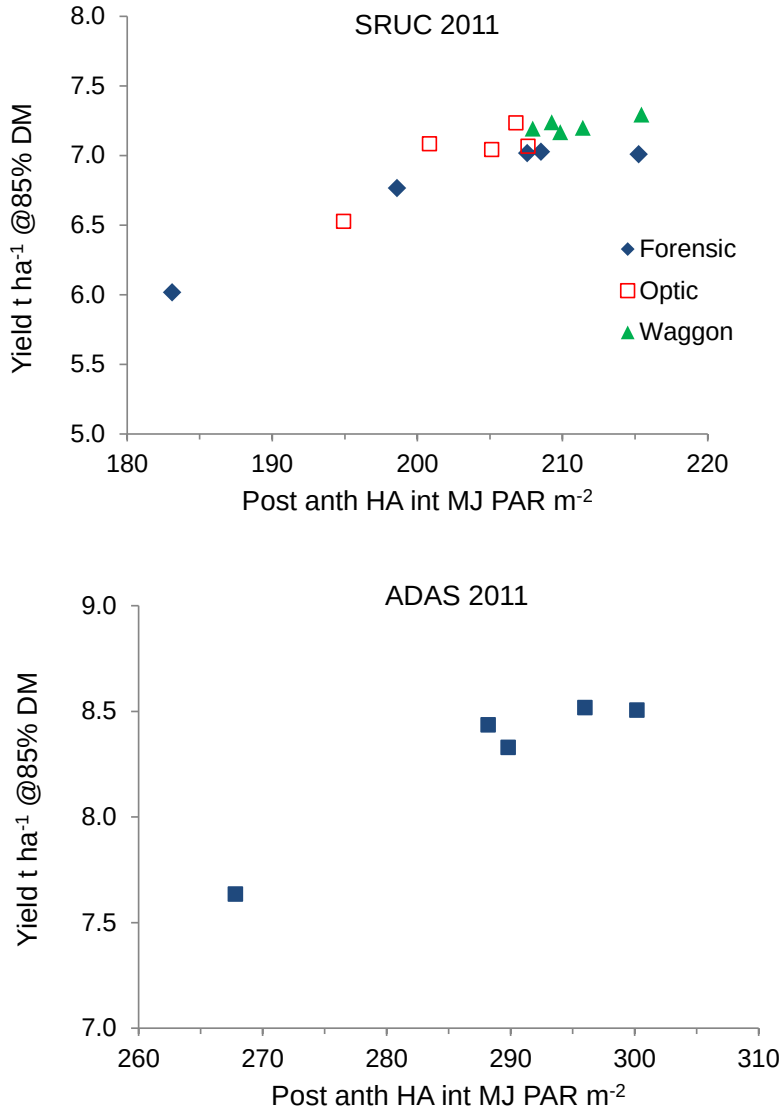


Fig. 7. Non-linear relationship between post-anthesis healthy area PAR interception and yield arising from fungicide timing treatments. At ADAS 2011 there was no significant interaction between variety and fungicide timing on yield and hence mean values across varieties are presented.

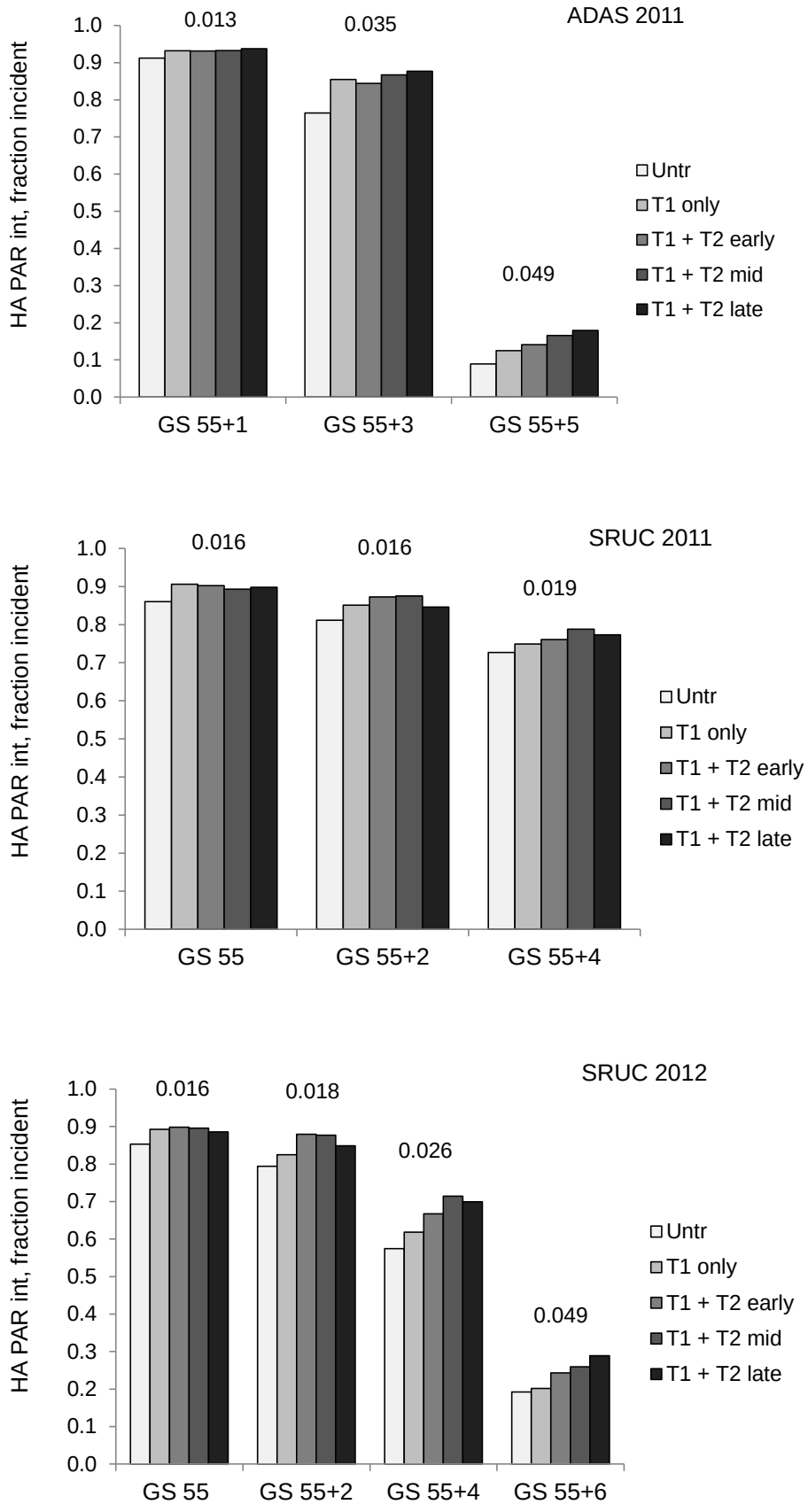


Fig. 8. PAR interception by healthy leaf area expressed as a fraction of the daily incident radiation at different stages during grain filling (weeks after GS55) and with different fungicide timings. Values above columns are the LSDs (5%) for main effects of fungicide treatment.

a significantly greater fraction than untreated crops. However, a second application at T2 had no additional effect at this growth stage. A significant benefit of a T2 application in protecting canopy PAR interception was observed from GS55+2 weeks at SRUC in 2012 where disease pressure was severe, but only from mid to late grain filling at ADAS and SRUC in 2011 (at GS55+5 weeks and GS55+4 weeks, respectively) where disease pressure was low or moderate. In general a T1 plus late T2 application gave more prolonged protection of the canopy and a greater total PAR interception compared to T1 plus early and mid T2 timings, but the differences only became apparent in the latter stages of grain filling (later than GS55+4 weeks) after appreciable canopy senescence had occurred (Fig. 8). This is beyond the critical period requiring protection identified from the shading experiments.

4.1.3. Conclusions

In summary, these experiments have shown that yield was sensitive to major reductions in light interception only during approximately the first 75% of grain filling. Where disease pressure was low or moderate, a T1 application on its own provided sufficient protection of light interception over this period and hence yield did not respond to a T2 application at either site in 2011. Where disease was more severe, a T2 application at the mid timing in addition to T1 was required to provide adequate protection during the critical 75% of grain filling period, and thus a yield response to a mid T2 timing was observed at SRUC in 2012. Later timings of T2 (after ear emergence) gave longer duration of canopy protection and PAR interception, irrespective of the disease pressure, but no significant yield improvement probably because the crop had sufficient assimilates available from storage reserves to supply the grain with carbon assimilates during the latter stages of grain filling (Gebbing *et al*. 1999; Bingham *et al*. 2007a).

4.2. Mechanisms by which fungicides increase grain numbers and yield in the absence of visible disease (objective 3).

4.2.1. Yield response to fungicide as a function of visible disease severity

When data from 12 separate experiments utilising resistant varieties were pooled, the yield response to fungicide did not relate well to the severity of visible disease recorded post-anthesis (Fig. 9). In each case the fungicide applications were prothioconazole plus pyraclostrobin (Proline plus Comet) at T1 and T2. Only a small proportion of the total variation in yield response was explained by variation in the disease severity of untreated plants (R^2 = 0.19) and the slope of the relationship was not significantly different to zero (P>0.05). There was little improvement when the reduction in disease severity from fungicide treatment (disease severity of untreated plants - severity of treated plants) was used as the explanatory variable (R^2 = 0.26). The y-intercept was significantly greater than zero (P<0.01) in each case, indicating that there was a significant overall

yield response to fungicide of around 0.4 t ha^{-1} in the absence of visible disease. This response is consistent with, though smaller than, that reported previously for selected lines from a spring barley doubled-haploid mapping population (Bingham *et al*., 2012a). The results also demonstrate that whilst on average there is a significant increase in yield in the relative absence of visible disease, the response can be inconsistent. In two experiments there was little or no response to fungicide in the absence of disease.

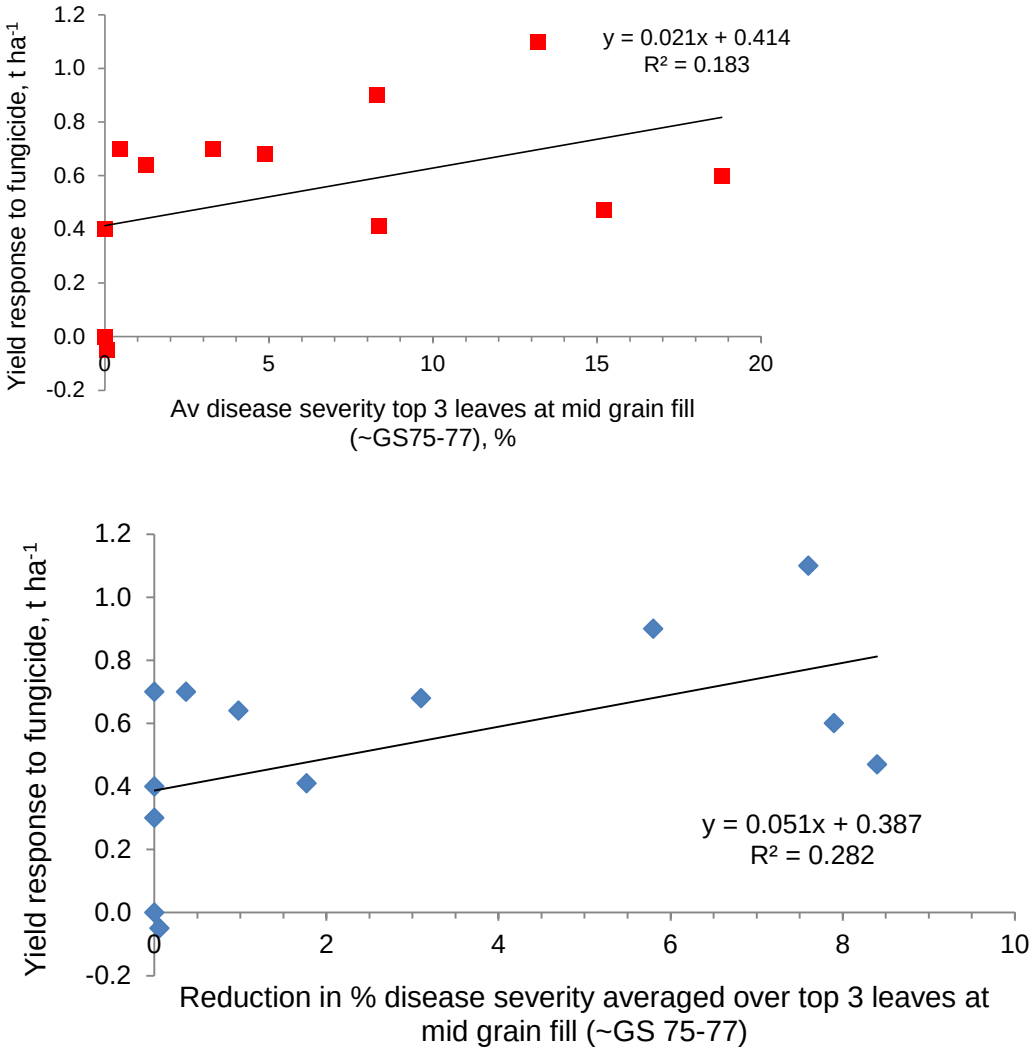


Fig. 9. Relationship between the yield response to fungicide (t ha^{-1} @ 85% DM) and the average disease severity of untreated plots, or the reduction in % disease severity resulting from fungicide treatment, assessed at mid grain filling. Each point represents the mean response from one of 12 separate experiments. All except two data points are values for the variety Westminster; two are the mean response of 3 disease resistant varieties. Lines fitted by least squares linear regression (slopes P>0.05; y-intercepts P<0.01)

In previous research, the yield response to fungicide was associated primarily with an increase in the number of grains m^{-2}, a yield component that is determined before flowering, yet there was

negligible disease and little effect on PAR interception by healthy tissue before anthesis. However, since the genotypes used in the previous work differed in disease susceptibility, we could not completely rule out the possibility that some disease was present, but went undetected, and that the crop is unusually sensitive to loss of green area during this phase of development. In the current project we have used host resistance to minimise disease development in order to investigate the response to fungicide in the absence or near absence of visible disease. Shading treatments were used to vary pre-anthesis light interception independently of fungicide and disease control so that the sensitivity of grain number formation and yield to loss of pre-anthesis healthy area PAR interception could be compared with the response to fungicide.

4.2.2. Effects of pre-anthesis shading

Little visible disease was observed before anthesis in all site-years. Significant disease developed after anthesis at ADAS 2011 (rhynchosporium average 14% over the top 3 leaves of untreated plants at GS77), but not the other sites-years. Fungicide treatment significantly ($P<0.01$) increased grain numbers m^{-2} at SRUC in 2009 in both shaded and non-shaded plots, but had no significant effect on pre-anthesis healthy area PAR interception. At this site there was, surprisingly, no effect of shading on grain numbers and thus the slope of the relationship between PAR interception and grain number was not significantly different to zero ($P>0.05$) in either fungicide treated or untreated plots (Fig. 10).

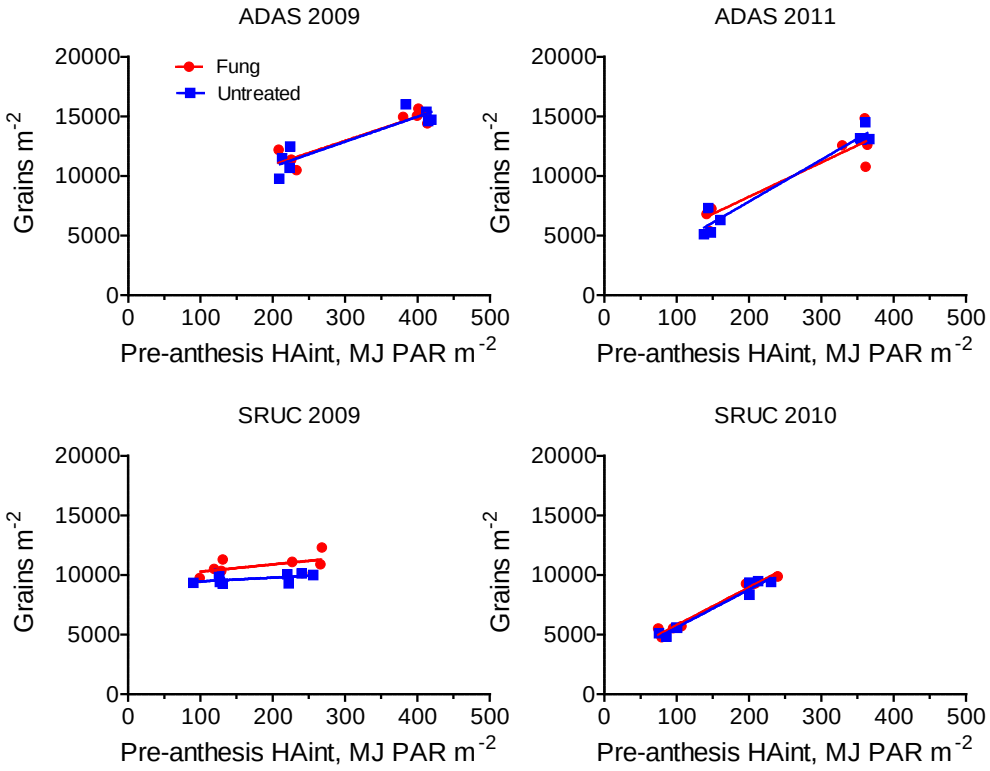


Fig.10. Relationship between pre-anthesis PAR interception by healthy tissue and grain number m^{-2} for fungicide treated and untreated crops. Shading was used to vary pre-anthesis PAR interception.

The failure of shading to reduce grain numbers at this site was the result of substantial secondary tiller production after the shades were removed; little secondary tillering occurred in non-shaded plots. The effect of shade removal on tillering may have been favoured by the availability of soil N late in the season as the experiment was sown after ploughing out a long term grass/clover ley.

There was no evidence of significant secondary tillering in the other site-years and at these sites shading reduced grain numbers substantially (regression $P<0.001$). Slopes and elevations of the regression equations did not differ significantly ($P>0.05$) between fungicide treated and untreated plots indicating that fungicide had no effect on the relationship between pre-anthesis PAR interception and grain number formation in these cases.

When data for non-shaded plots at SRUC 2009 and shaded and non-shaded plots for the other site years were pooled (i.e. treatments and sites where there was little secondary tillering), a single linear regression described well ($R^2 = 0.89$) the relationship between pre-anthesis healthy area PAR interception and grain number (Fig. 11).

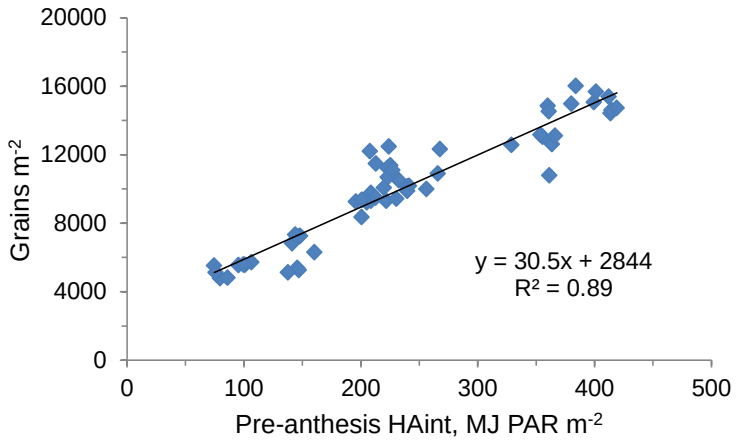


Fig. 11. Relationship between pre-anthesis PAR interception by healthy tissue and grain numbers m^{-2} for data pooled from different site years, fungicide and shading treatments, excluding those (i.e. shaded plots at SRUC 2009) where significant secondary tillering occurred. Line fitted by linear regression ($P<0.001$).

The slope of 30.5 grains MJ^{-1} PAR over a wide range of healthy area PAR interception values implies that an extra 32 MJ m^{-2} PAR must be intercepted for every 1000 additional grains m^{-2} produced. We can estimate that for fungicide treatment to increase grain numbers by 1000 through

the control of visible disease and improvement in pre-anthesis healthy area PAR interception that the untreated canopy would need to have an average disease severity of ~18% over the laminae of the top 5 leaves from the start of stem extension to ear emergence. These estimates are based on the canopy characteristics of the high yielding crop at ADAS 2011 (average yield of non-shaded plots 7.4 t ha^{-1} @ 85% DM). They assume that disease is located only on the leaf laminae and that it is distributed evenly across the leaf layers. In reality, during stem extension foliar disease tends to be located mostly in the lower canopy where it has relatively less effect on PAR interception. Thus the value of 18% average disease severity is likely to be an underestimate of the severity required. It is inconceivable, therefore, that where increases in grain numbers of this magnitude are found in the apparent absence of visible disease (e.g. SRUC 2009) that it is the result of a failure to observe and quantify disease severity accurately.

These experiments have demonstrated that treatment of crops with prothioconazole plus pyraclostrobin can, in some situations (i.e. SRUC 2009), increase grain numbers where there is minimal visible disease pre-anthesis. The increase was not associated with a significant increase in healthy area light interception pre-anthesis and so we cannot ascribe it to the control of small amounts of visible disease or the effects of fungicide on leaf area expansion. Alternative explanations are that fungicides increase grain number formation by controlling pre-anthesis asymptomatic infection by endophytic pathogens, by controlling epiphytic saprophytes on the leaf surface, or by modifying host metabolism and tiller or ear development. In order to test these hypotheses, a series of experiments were conducted in which different fungicide chemistries were compared.

4.2.3. Response to contrasting fungicide chemistries

Yield and yield components

This project has shown that fungicide application can increase yield and grain numbers m^{-2}, where visible disease is low or absent, however, the response can be inconsistent. A cross-site anova of experiments comparing the response to fungicide chemistries was conducted to improve the power of the analysis in order to gain a clearer insight into the possible mechanisms underlying these responses. In the analysis, site-years was treated as a random effect.

Table 3. Effects of fungicide products with contrasting chemistries on the yield and yield components of spring barley cv. Westminster. Values are means from a cross-site ANOVA of experiments at SRUC Aberdeen, SRUC Edinburgh and ADAS in 2012.

Fungicide	Yield t ha^{-1} @85% DM	Ears m^{-2}	Grains m^{-2}	MGW, mg @85% DM
Bravo	5.61	910	12979	43.50
Comet	5.71	947	13443	42.94
Proline	5.71	906	13355	43.16
Proline + Comet	5.89	968	13678	43.54
Untreated	5.25	937	12718	41.62
P	<0.001	0.274	0.050	0.064
LSD (5%)	0.202	63.4	678.6	1.444

In 2012, the effects of chlorothalonil (Bravo), prothioconazole (Proline), pyraclostrobin (Comet) and prothioconazole plus pyraclostrobin (PC) were compared on the variety Westminster at three sites. All fungicide groups increased yield significantly compared to untreated controls (Table 3). The increase was greatest (12%) with Proline plus Comet and least (7%) with Bravo. By contrast Bravo and Proline plus Comet increased mean grain weight (MGW) to a similar extent (4.5%); an effect close to statistical significance at the 5% level. Thus the greater effect of Proline plus Comet on yield compared to Bravo was the result of the impact on grain number m^{-2}. Proline plus Comet increased grain numbers m^{-2} by nearly 1000 relative to untreated controls (a 7.5% increase), whilst there was no significant effect of Bravo. When applied separately, Proline and Comet were equally effective at increasing grain numbers. None of the treatments influenced the number of ears m^{-2}.

A separate cross-site analysis was conducted on data from experiments at ADAS and SRUC in 2009–2011 with identical results. These experiments were conducted on the variety Westminster and had common core treatments of Bravo, Proline plus Comet and untreated controls. As found in 2012, both Bravo and Proline plus Comet increased MGW to the same extent, but only Proline plus Comet significantly increased grain number m^{-2} relative to controls (Table 4). The fact that identical results have been found from separate cross-site analyses of two independent data sets means that we can be confident that the contrasting chemistries of Bravo and Proline plus Comet are generating different biological responses within barley.

Table 4. Effects of fungicide products with contrasting chemistries on the yield and yield components of spring barley cv. Westminster. Values are means from a cross-site anova of experiments at ADAS 2009 and 2011 and SRUC Edinburgh 2010 and 2011. Site-year was analysed as a random effect.

Fungicide	Yield t ha^{-1} @85% DM	Grains m^{-2}	MGW, mg @85% DM
Bravo	6.98	13320	52.82
Proline + Comet	7.28	13834	52.90
Untreated	6.68	13240	51.00
P	<0.001	0.025	0.001
LSD (5%)	0.228	455.2	1.069

A more complete set of physiological measurements were made in the 2012 experiments than those in 2009–2011 and thus the analysis of mechanisms underlying the contrasting yield responses to different fungicide chemistries focussed on the 2012 experiments.

Disease, GLA and healthy area PAR interception

In spite of its good resistance to several major barley pathogens, some foliar disease did develop on Westminster in these experiments. The main disease at the SRUC sites in Aberdeen and Edinburgh was ramularia, with some rhynchosporium also present. At ADAS the main disease was rhynchosporium; in addition there was some physiological spotting. The severity of each disease and disorder was summed for each leaf layer and analysed as total disease.

Table 5. Effects of fungicide products with contrasting chemistries on the total disease severity and green leaf area (GLA) percentage of spring barley cv. Westminster. Values are means across the top 4 leaves except at GS75 where they are averaged over the top 3 leaves and are from a cross-site ANOVA of experiments in 2012. Data were arcsine transformed prior to analysis; back-transformed means are presented. Means within a column followed by a different letter are significantly different at P<0.05.

	Disease total, %					GLA, %				
	GS 39	GS 59		GS 75		GS 39		GS 59	GS 75	
Bravo	0.06	0.22	a	2.94	a	99.6	a	91.9	66.8	a
Comet	0.07	0.40	a	4.79	b	99.4	ab	92.3	61.6	b
Proline	0.04	0.38	a	2.90	a	99.6	a	91.2	68.5	a
Proline + Comet	0.06	0.30	a	3.02	a	99.2	b	93.1	69.8	a
Untreated	0.04	0.65	b	6.68	c	99.6	a	91.5	58.1	b
P	0.893	0.004		<0.001		0.049		0.613	<0.001	

There were only trace amounts of disease at GS39 and no significant effect of fungicide treatment (Table 5). Although there was a significant overall effect of fungicide treatment on green leaf area (GLA) at GS39, the differences were trivial (<0.5% GLA). At GS59, a small amount of disease was present in untreated plots and there was a significant and comparable reduction with each fungicide product; however, GLA did not differ at this growth stage. By GS75, disease severity had increased to nearly 7% (over the top 3 leaves). Bravo, Proline and Proline plus Comet gave similar levels of disease control (difference in disease severity between treatment and untreated control) and similar values of GLA (Table 5). By comparison, Comet was less effective in controlling visible disease and increasing GLA.

There was no significant difference between treatments in the amount of PAR intercepted by healthy tissue before anthesis (which is assumed to coincide with ear emergence) (Table 6). This is consistent with the negligible amount of disease and insignificant effects of fungicide on GLA found at, and before, flowering. The results also indicate that the fungicide chemistries did not influence pre-anthesis canopy growth in a way that modified PAR interception. Since Proline plus Comet increased grain numbers, but not pre-anthesis PAR interception, there was an increase of around 5 grains MJ^{-1} of PAR interception compared to untreated controls. By contrast, Bravo did not significantly increase the number of grains produced MJ^{-1} of PAR interception. The increase with Proline plus Comet is the same (an additional 5 grains MJ^{-1}) as that reported previously in response to another triazole plus strobilurin fungicide programme based on prothioconazole (Bingham *et al*., 2012a). Both Proline and Comet increased grains per MJ^{-1} to a similar extent (3-4 grains), but were less effective applied separately than when used in combination.

Table 6. Effects of fungicide products with contrasting chemistries on the amount of PAR intercepted by healthy tissue (HAint) pre- and post-anthesis. Grains MJ^{-1} is given by the final grain number divided by the amount of pre-anthesis HAint. Values are means from a cross-site anova of experiments in 2012.

	HAint, MJ PAR m^{-2}			
	Pre-anth	Post-anth	Total	Grains MJ^{-1} pre-anth
Bravo	254	192	442	52.4
Comet	252	187	435	54.7
Proline	255	190	441	54.0
Proline + Comet	252	192	441	56.0
Untreated	253	177	427	51.3
P	0.750	<0.001	<0.001	0.014
LSD (5%)		5.8	6.7	2.82

All fungicide treatments resulted in a significant increase in PAR interception by healthy tissue after anthesis (Table 6), which is in line with their effects on the control of visible disease and protection of GLA during grain filling (Table 5).

Asymptomatic pathogen infection and saprophytic leaf surface fungi

There was no significant difference between fungicide-treated and untreated plots in the amount of ramularia and rhynchosporium DNA in leaf extracts at GS39 (Table 7). By GS59 the amount of pathogen DNA had increased in untreated plots and all fungicide products gave a significant reduction relative to these controls. There was no significant difference between contrasting fungicide chemistries on the amount of pathogen DNA in leaf extracts. These results are consistent with those presented for total visible disease severity in Table 5.

A new PCR assay was developed to quantify the amount of saprophytic fungal DNA belonging to the genus *Cladosporium*. Flag leaves were sampled at GS39 and GS59 from the experiment at SRUC Edinburgh 2012 checked for the absence of visible disease lesions and washed to remove leaf surface (epiphytic) microorganisms. Although the results are quite variable they indicate a significant reduction in *Cladosporium sp* DNA in leaf washings with Bravo and Proline compared to untreated controls (Fig. 12). Comet also reduced *Cladosporium sp* DNA, but to a smaller and not statistically significant extent. At ADAS 2012, leaf 2 (leaf below flag leaf) was sampled from the same treatments at GS59 for microscopic examination of the leaf surface. These leaves were without visible disease lesions. Major reductions (>60%) in the density of hyphae in lamina and mid-rib regions were observed with each of the fungicide chemistries compared to untreated controls. Overall, Bravo and Proline were significantly more effective at reducing hyphal density than Comet, especially in the lamina regions. In addition, all fungicide chemistries significantly reduced the density of spores found in laminae regions, although not along the mid-rib. Given the absence of visible disease symptoms in these samples, the fungal hyphae are likely to belong to species of epiphytic saprophytes. As such the results of the microscopic examination at ADAS are consistent with the quantification of *Cladosporium sp* DNA at SRUC Edinburgh, in that both sets of measurements highlight significant reductions in growth of fungi on the surface of leaves treated with Bravo and Proline and, to a lesser extent, Comet.

Table 7. Quantities of ramularia and rhynchosporium DNA (pg 100 ng^{-1} total DNA) in extracts of bulked leaf samples from the top 4 leaf layers determined by qPCR. Results are from a cross-site analysis of experiments in 2012 where site was analysed as a random effect. Data were transformed [log10 (x+1)] prior to analysis and values are back-transformed treatment means; *LSDs are the transformed values. Within a column, means followed by a different letter are significantly different at P<0.05.

	Ramularia			Rhyncho		
	GS 39	GS 59		GS 39	GS 59	
Bravo	1.88	1.32	a	0.08	0.21	a
Comet	2.49	3.54	a	0.06	0.56	a
Proline	1.43	2.24	a	0.05	0.19	a
Proline + Comet	0.84	1.96	a	0.30	0.17	a
Untr	1.29	9.91	b	0.53	2.55	b
P	0.385	<0.001		0.142	0.017	
LSD (5%)*	ns	0.292		ns	0.319	

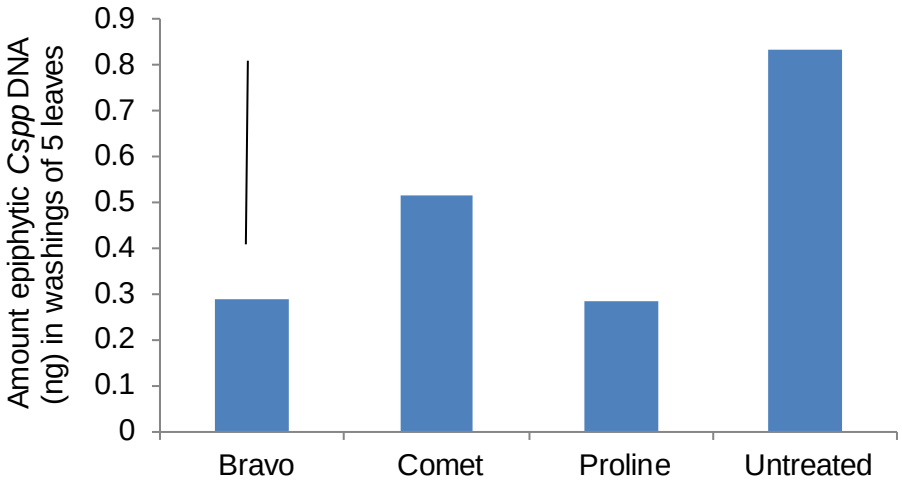
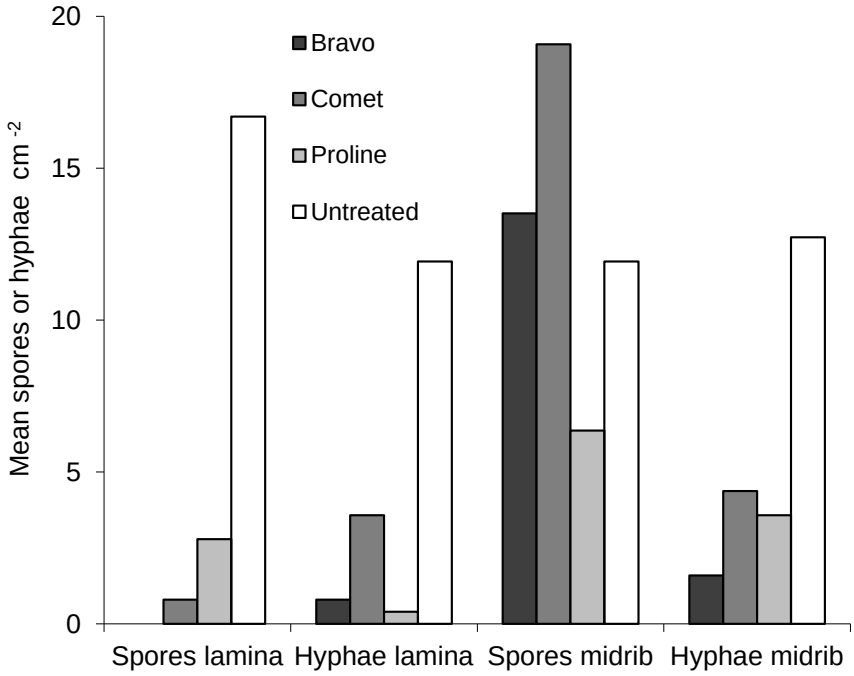


Fig. 12. Amount *Cladosporium sp* DNA in washings of flag leaves treated with different fungicide products. Data are from samples taken at GS39 and GS59 from cv. Westminster at SRUC Edinburgh 2012. Values are means across sample growth stages (GS); P=0.034 for fungicide (F); F*GS, ns. Vertical bar is the LSD (5%) for effects of fungicide.



F Spores leaf <0.001, LSD 0.53 (df 153)
F Hyphae leaf <0.001, LSD 0.347 (df 153)
F Hyphae mid-rib <0.001, LSD 0.318 (df 153)
Spores mid-rib, ns

Fig.13. Densities of fungal spores and hyphae on the surface of leaf 2 (one below flag leaf) at GS59 following treatment with different fungicide products. LSD values at the 5% level.

4.2.4. Possible mechanisms determining yield increase

The results indicate that there were two components to the yield increases observed with fungicide treatments. The first involved an increase in grain numbers m^{-2} and was elicited by Proline and Comet (alone and in combination), but not Bravo. The second involved an increase in MGW and was elicited by both Bravo and Proline plus Comet; effects of Proline and Comet on MGW were smaller when applied separately. By comparing the relative impact of these fungicide chemistries on visible disease, asymptomatic pathogen infection, and healthy area PAR interception in relation to key phases of crop development, it is possible to disentangle some of the mechanisms contributing to the overall yield response. For clarity, the following discussion focusses on a comparison of the Bravo and Proline plus Comet treatments, but it should be emphasised that both the triazole and strobilurin components of the Proline plus Comet mixture contributed to the response.

As grain numbers are determined by the production and survival of tillers and spikelets they are influenced, for the most part, by events occurring before anthesis. In some circumstances, secondary tillering can occur after anthesis, as was found at SRUC in 2009 following the removal

of pre-anthesis shades, however, this is exceptional and, under standard crop management conditions, significant secondary tillering is rare. The grain number m^{-2} response to Proline and Comet must, therefore, be elicited prior to flowering. The response occurred in the absence of any increase in healthy area PAR interception before anthesis and hence was not the result of the control of visible disease and an increase in green area. Since Bravo and Proline plus Comet resulted in comparable reductions in ramularia and rhynchosporium DNA in leaf extracts by anthesis, but only Proline plus Comet, increased grain numbers, the response cannot be ascribed to the control of asymptomatic endophytic infection by the two major pathogens. Similarly, we have evidence that both Bravo and Proline, and to a lesser extent Comet reduced the amount of fungal hyphae observed on the leaf surface and the abundance *Cladosporium* sp. in leaf washings when measured in independent experiments at separate sites. Although the effects of Proline *plus* Comet were not determined in these experiments, it seems unlikely that the control of leaf surface saprophytes is the explanation for the grain number response. Bravo had no significant effect on grain numbers, but resulted in a large and significant reduction in abundance of leaf surface fungi, which suggests that grain number formation is not sensitive to the control of epiphytes.

Although we have no direct evidence of an effect of Proline and Comet on host metabolism in the current study, through the elimination of other candidate mechanisms, a direct effect on the physiology of the plant is the most plausible explanation for the increase in grain numbers observed. This would be consistent with reports of triazoles interfering with gibberellin biosynthesis (Rademacher, 2000) and strobilurins modifying ethylene and cytokinin metabolism (Grossman *et al.,* 1999). In addition, there is evidence that triazoles and strobilurins may reduce oxidative stress within plant tissues (Wu and Tiedemann, 2001). However, it is important to emphasise that most of the earlier reports of effects on plant metabolism have been associated with delays in leaf senescence post-anthesis. Our results suggest that a triazole and strobilurin programme may increase grain numbers by modifying plant metabolism *prior* to anthesis and in a way that is independent of effects on leaf senescence since pre-anthesis healthy area PAR interception was not affected.

Under low to moderate disease conditions, Bravo and Proline plus Comet protected the canopy equally well during grain filling and resulted in comparable increases in healthy area PAR interception and MGW relative to untreated controls when averaged across sites (Tables 3 and 6). However, since Proline plus Comet also increased the number of grains m^{-2} in these experiments, there was a larger grain sink to supply with assimilate during grain filling. So given that the interception of PAR required to drive photosynthesis during grain filling was the same in Bravo and Proline plus Comet treated crops, where did the additional assimilate come from to satisfy the larger grain sink in the latter treatment? There are two possibilities. Firstly, Proline plus Comet may have increased radiation use efficiency (RUE) relative to Bravo treated crops so that the canopy

produced a greater amount of dry matter per MJ of PAR intercepted during grain filling. Secondly, Proline plus Comet treated crops may have utilised a larger quantity of pre-anthesis storage reserves compared to those treated with Bravo. This is evident in Fig. 14 in which data have been pooled from several experiments. Here the yield response to fungicide (treated-untreated yield) is plotted against the increase in post-anthesis healthy area PAR interception resulting from fungicide treatments. The slopes of the relationships for Proline plus Comet and Bravo were small and not significantly different from zero. This implies that the yield response was not particularly sensitive to the increase in post-anthesis PAR interception when compared across sites. This might occur if RUE or the utilisation of storage reserves were restricted at those sites where there was a large increase in PAR interception with fungicide treatment, possibly as a result of a limited grain storage capacity and hence demand for assimilate. Reductions in post-anthesis RUE towards the end of grain filling have been observed in barley crops where there is a relatively large source-sink ratio (Bingham *et al.,* 2007a). Furthermore, experiments reported in the current project (section 4.1.2) have shown that fungicides can prolong post-anthesis green area retention and increase PAR interception beyond that required to maximise yield at a given site-year.

Elevations (y-intercepts) of the relationships of yield response on PAR interception increase were significantly different ($P<0.001$), highlighting a greater increase in yield for any given increase in PAR interception following treatment with Proline plus Comet compared with Bravo. This is indicative of an increase in RUE and/or greater allocation of storage reserves to grain yield after treatment with Proline plus Comet that is consistent across sites.

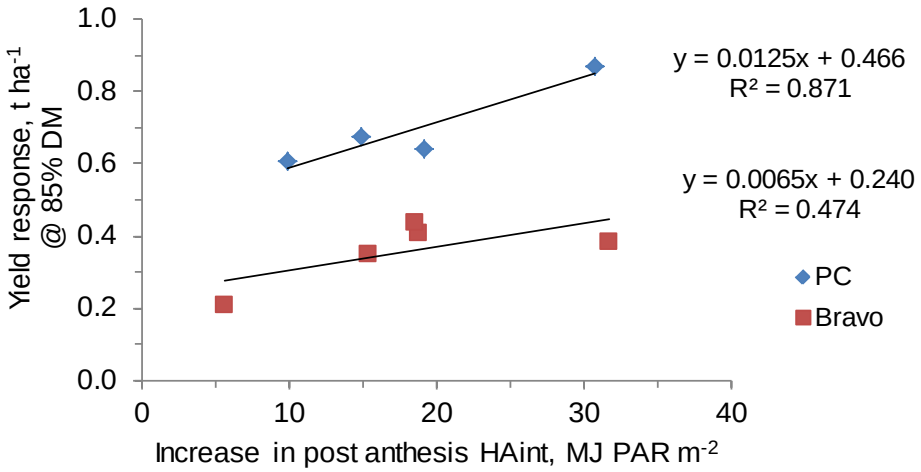


Fig. 14. Relationship between the increase in post-anthesis PAR interception by healthy tissue resulting from fungicide application and the increase in yield of cv. Westminster. Data are treatments means from experiments at SRUC 2010, 2012, ADAS 2011, 2012.

The analysis of effects of fungicide chemistry on grain development and growth is complicated by the fact that dry matter produced from a given amount of resource captured (PAR) must be distributed over a larger number of grains following treatment with Proline plus Comet compared with Bravo. Moreover, there is variation between site-years in the number of grains produced. Therefore, in order to examine the impact of the different fungicide chemistries on MGW more closely it is useful to express the values of post-anthesis PAR interception per unit grain number. This normalises the data for the variation in grain numbers. When the increase in PAR interception per grain was plotted against the increase in MGW resulting from fungicide application a positive relationship was found indicating that MGW increased when fungicide increased healthy area PAR interception per unit grain number post-anthesis (Fig. 15). The slope of the relationship was the same for Bravo and Proline plus Comet treated crops (slopes not significantly different), but the elevations were significantly different ($P<0.05$) and for Proline plus Comet the line was displaced to the left. Thus across site-years a given increase in MGW with Proline plus Comet was achieved with a smaller increase in PAR interception per unit grain number. This further illustrates effects of Proline plus Comet beyond those on the protection of green tissue and PAR interception; effects that must involve an increase in RUE or utilisation of storage reserves in grain filling relative to crops treated with Bravo.

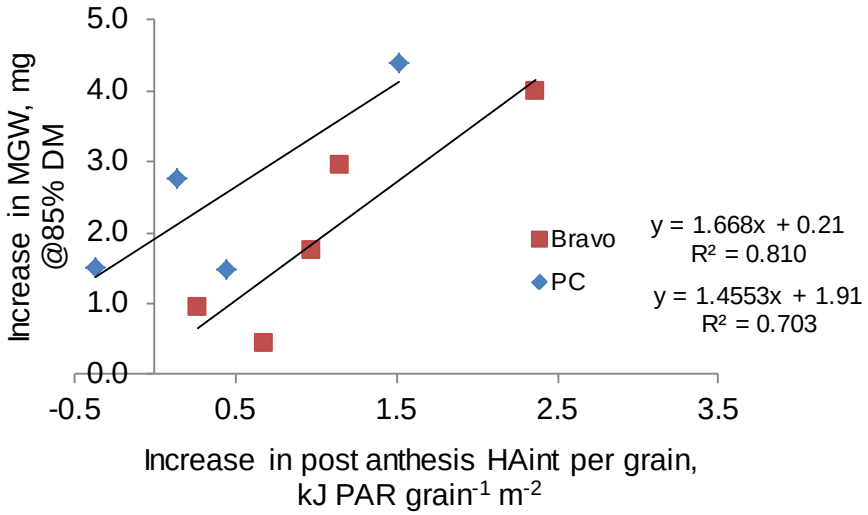


Fig. 15. Relationship between the increase in PAR interception by healthy tissue post-anthesis (expressed per unit grain number) and the increase in MGW in response to fungicide treatment.

Pre-harvest samples were taken at SRUC Aberdeen and Edinburgh sites in 2012 for determination of total biomass, harvest index (HI) and tissue N concentrations. The results are presented along with an analysis of N use efficiency in section 4.5. Based on just these two sites, the increase in yield from Proline plus Comet compared with Bravo was approximately 6%, total biomass was increased by 7%, RUE by 6% and there was no significant difference in HI (Table 21). These

results support the view that the additional biomass required for filling the larger number of grains in Proline plus Comet treated crops came from a greater RUE rather than a greater utilisation of storage reserves. Moreover, since the control of visible disease and healthy area PAR interception was the same in Bravo and Proline plus Comet treated plants, the increase in RUE was unlikely to arise from the control of pathogen infection. As the estimates of RUE were based on season-long measurements of PAR interception and final crop biomass, it is not possible to determine whether the increase in RUE following treatment with Proline plus Comet occurred pre-anthesis and was causally related to the increase in grain number formation. However, such an effect is plausible mechanistically.

In the 2012 experiments, significant visible disease developed post-anthesis, even though the resistant variety Westminster was grown. The improvements in post-anthesis healthy area PAR interception with Bravo and Proline plus Comet in 2012 were, therefore, associated with the control of visible disease. However, in other experiments evidence was found of an increase in green leaf area following treatment with fungicide in the absence of disease (Table 8). In these cases, the delay in leaf senescence could have been the result of the control of leaf surface saprophytes (Fig. 12; Smedegaard-Petersen and Tolstrup, 1985) or reductions in oxidative stress (Wu and Tiedemann, 2001). However, in those site-years where there was little visible disease (ADAS 2009 and SRUC 2010) increases in MGW with fungicide application were only small and not statistically significant. Thus, there is no evidence from the current project to link the prolonged green area in the absence of disease with an increase in MGW. A greater number of site-years would be required to test whether there is a significant association. The potential mechanisms underlying the yield responses to fungicide are summarised in Table 9.

Table 8. Effects of fungicides on post-anthesis disease severity and % GLA in different site-years. Values are means across the top 3 leaves made at ~GS75–77 on cv. Westminster.

	SRUC 2010		ADAS 2009		ADAS 2011	
	% GLA	% Disease	% GLA	% Disease	% GLA	% Disease
Untreated	67.2	0	43.7	0.452	59.9	9.5
Bravo	82.3	0	67.0	0.078	84.7	3.0
Proline plus Comet	77.1	0	88.7	0.076	84.8	3.0
LSD (5%)	11.4	ns	10.65	ns	9.69	1.14

Table 9. Summary of mechanisms by which fungicides increased yield. Hyphen indicates measurements required to provide evidence were not made. PC refers to Proline plus Comet.

Candidate mechanism	*Increase in grain no. m^{-2}, determined pre-anthesis*	*Increase in MGW, determined around and post-anthesis*	
	PC	*PC*	*Bravo*
Control of visible disease	No	Yes	Yes
Increase in PAR interception	No	Yes	Yes
Increased RUE	Possible	Yes	No
Control of asymptomatic infection	No	-	-
Control of leaf surface saprophytes	No	Possible	Possible
Direct effects on plant metabolism	Likely	-	-
Increased leaf lifespan independent of disease control	No	Possible	Possible

4.3. Effects of fungicide timing on yield and sink components in disease resistant varieties (objective 4)

Previous experiments designed to investigate the effects of fungicides on yield formation in the relative absence of disease have focussed on Westminster as the sole disease resistant variety. The aim of the experiments reported in this section was to determine whether similar responses are found in other disease resistant varieties and whether the timing of fungicide application is important in influencing the response. Here, the yield and yield components of three varieties, Westminster, Quench and Garner were compared after treatment with Proline plus Comet at T1, (GS30–31), T2 (GS45–49) or T1 and T2.

4.3.1. 2011 Experiments

In 2011, experiments at SRUC and ADAS involved different designs, consequently the data for each site have been analysed separately. As there were few variety by fungicide interactions in yield and yield components, only the means for fungicide treatment averaged across varieties are presented (Table 10).

At each site yields did not differ significantly between varieties (Table 10), although Quench produced larger numbers of smaller grains than Garner and Westminster. There was a significant increase in yield at SRUC in 2011 of between 0.29 and 0.41 t ha^{-1} with fungicide treatment when averaged across varieties. There was no significant difference between the fungicide timings. The

yield increases were associated with small, though not statistically significant, increases in grain numbers and significant increases in MGW. As there was no significant variety by fungicide interaction, the observed yield responses to fungicide were consistent across the three varieties. The main disorder in this experiment was physiological leaf spotting, which was most pronounced on cv. Garner, and there was no effect of fungicide on disease/disorder severity and GLA at anthesis or during grain filling (Table 11). The yield responses were, therefore, found across varieties in the absence of control of visible disease and protection of green leaf area.

At ADAS in 2011, the yield response to fungicide was greater, ranging from 0.96 to 1.11 t ha^{-1}. This was associated with significant increases in both grain numbers and MGW. Significant visible disease (rhynchosporium) had developed by ear emergence (GS59) and increased in severity during grain filling (GS75). Fungicide timings differed in how well they controlled disease and protected GLA. At GS75 the T1 plus T2 timing resulted in a significantly larger GLA and marginally less disease than the T1 or T2 applications on their own. However, yield and grain number responses did not differ significantly between the different fungicide timings, although the increase in MGW was significantly greater following a T1 plus T2 application than with either on its own. There was no significant variety by fungicide interaction on disease or GLA when disease severity was at its greatest (i.e. GS75).

Table 10. 2011 yield and yield components of disease resistant varieties treated with Proline plus Comet at T1, T2 and both T1 and T2; Untr refers to untreated controls. Values are means across the three varieties. P values are for V (variety), F (fungicide timing) and the V*F interaction.

	SRUC 2011 @85% DM			ADAS 2011 @85% DM		
	Yield	Grains m^{-2}	MGW	Yield	Grains m^{-2}	MGW
Untr	6.52	12724	51.40	7.83	16371	47.96
T1	6.81	12942	52.82	8.79	17330	50.83
T2	6.88	13120	52.69	8.67	17204	50.57
T1 + T2	6.93	13087	53.19	8.94	17301	51.78
V	ns	<0.001	<0.001	ns	<0.001	<0.001
F	0.011	ns	0.047	<0.001	0.039	<0.001
V*F	ns	ns	ns	ns	ns	0.021
F LSD (5%)	0.250		1.289	0.345	742.2	0.832

Table 11. Visible disease severity (sum of all diseases and disorders averaged over the top 3 leaves) and % GLA (averaged over the top 4 leaves) following different fungicide timings on disease resistant varieties at SRUC and ADAS in 2011. Values were arcsine transformed prior to analysis. Back-transformed mean values of fungicide effects averaged across varieties are presented. Within a column, values followed by a different letter are significantly different at P=0.05. Plots were treated with Proline plus Comet at T1, T2 or both T1 and T2; Untr refers to untreated controls.

	SRUC 2011				ADAS 2011							
	GS 59		GS 75		GS 59				GS 75			
	% Disease	% GLA	% Disease	% GLA	% Disease		% GLA		% Disease		% GLA	
Untr	0.31	94.6	6.54	63.1	7.12	a	58.3	a	10.95	a	12.2	a
T1	0.19	94.5	6.31	65.8	1.37	b	79.1	bc	9.52	ab	29.5	b
T2	0.12	95.2	4.92	70.2	3.12	c	74.6	b	7.29	b	36.3	b
T1 + T2	0.14	95.4	5.41	70.5	0.79	b	82.7	c	5.10	b	44.5	c
V	0.324	0.366	0.015	<0.01	<0.001		<0.001		0.018		<0.001	
F	0.479	0.754	0.136	0.134	<0.001		<0.001		0.001		<0.001	
V*F	0.512	0.614	0.557	0.118	0.025		0.068		0.365		0.442	

4.3.2. 2012 Experiments

In 2012, experiments were conducted at three sites under a common split-plot design. A cross-site analysis of the data was carried out, using site as a random effect. Yields were low at all three sites in 2012 largely because of the exceptionally wet weather and low incident PAR. There were highly significant effects of fungicide treatment on yield, grain numbers and MGW, but not on the number of ears m^{-2} (Table 12). There was no significant interaction between fungicide treatment and variety indicating that the response of each variety was comparable. In general yields, grain numbers and MGW were increased more when treated with a T2 application either alone or in combination with T1 than when treated with a T1 application on its own. There was no significant difference between the T2 and T1 plus T2 treatments on yield, grain numbers and MGW.

Table 12. 2012 yield and yield components of disease resistant varieties treated with Proline plus Comet at T1, T2 and both T1 and T2; Untreated refers to non-fungicide treated controls. Values are means across the three varieties in a cross site analysis with site as a random effect. P values are for V (variety), F (fungicide timing) and the V*F interaction.

	Yield, t ha^{-1} @ 85% DM	Grains m^{-2}	MGW, mg @ 85% DM	Ears m^{-2}
Untreated	5.20	12978	40.54	937
T1	5.53	13295	42.11	964
T2	5.84	13818	42.69	968
T1 + T2	5.89	13897	42.90	977
V	0.264	<0.001	<0.001	0.002
F	<0.001	<0.001	<0.001	0.189
V*F	0.781	0.932	0.694	0.553
F LSD (5%)	0.133	406.9	0.698	ns

At both SRUC sites the main disease present was ramularia, with a small amount of rhynchosporium observed during grain filling at SRUC Aberdeen. At ADAS there was some brown spotting visible before and after flowering. The main disease rhynchosporium, however, was negligible or absent until after flowering. In the cross-site analysis, total disease was negligible at and before GS59, although small and significant reductions were found with T2 fungicide applications compared to T1 and untreated controls (Table 13). Disease severity increased between GS59 and GS75 and all fungicide timings reduced visible disease significantly at GS75, but the most effective was the T1 plus T2 treatment. Compared to T1 applications and controls, the greater disease control resulting from the T2 and T1 plus T2 applications was associated with a significantly greater GLA (Table 13). Although there were some significant interactions between variety and fungicide timing, they were not consistent across growth stages and related to differences in the severity of disease or the GLA of untreated controls in the different varieties. In general there was less disease on untreated Westminster than the other two varieties (data not shown).

Table 13. Total visible disease severity (sum of all diseases and disorders averaged over the top 4 leaves, except GS75 when only top 3 leaves included) and % GLA (averaged over the top 4 leaves) following different timings of Proline plus Comet on 3 disease resistant varieties. Results are from a cross-site analysis of experiments in 2012 using site as a random effect. Values were arcsine transformed prior to analysis. Back-transformed mean values are presented for effects of fungicide treatment averaged over varieties. Within a column values followed by a different letter are significantly different at P=0.05.

	Total disease, %					GLA, %				
	GS 39	GS 59		GS 75		GS 39	GS 59		GS 75	
Untreated	0.19	0.95	a	8.66	a	99.2	89.6	a	53.3	a
T1	0.17	0.84	a	5.69	b	99.3	92.3	b	61.2	b
T2	0.16	0.58	b	4.32	c	99.2	92.7	b	65.6	c
T1 + T2	0.16	0.52	b	3.41	d	99.2	93.3	b	67.4	c
V	0.012	0.018		0.01		0.445	0.212		<0.001	
F	0.91	<0.001		<0.001		0.913	<0.001		<0.001	
V*F	0.018	0.776		0.062		0.104	0.013		0.765	

Table 14. Effects of applying Proline plus Comet at different timings on the quantity of pathogen DNA (pg DNA (100 ng)$^{-1}$ total DNA) in extracts from bulked leaf samples. Pathogen DNA was determined by qPCR on extracts from samples taken from the top 4 leaf layers. Values are fungicide treatment means from a cross-site analysis of experiments in 2012 with site as a fixed effect. Within a column, values followed by a different letter are significantly different at P=0.05. P values for main effects of site (S), variety (V) and fungicide timing (F) and their interactions are given; ns, effect not significant.

	Ramularia			Rhyncho		
	GS 39	GS 59		GS 39	GS 59	
Untreated	1.39	17.49	a	0.64 ab	8.12	a
T1	1.69	5.47	b	0.17 b	1.37	b
T2	1.49	3.56	c	1.00 a	1.19	b
T1 + T2	1.10	1.70	d	0.17 b	0.22	c
S	ns	<0.001		0.074	0.030	
V	ns	0.038		ns	0.036	
S*V	ns	ns		ns	ns	
F	ns	<0.001		0.005	<0.001	
S*F	ns	<0.001		0.063	0.045	
V*F	ns	ns		ns	0.004	
S*V*F	ns	ns		ns	ns	

The amount of ramularia and rhynchosporium DNA was determined by qPCR on bulked leaf samples taken from the top 4 leaf layers. As rhynchosporium was the major visible disease at ADAS and ramularia at SRUC, strong site effects and interactions between site, variety and fungicide treatment were anticipated. Thus the data were analysed using site as a fixed effect in order to explore these possible interactions. The full analysis is given in Appendix 4. For clarity of presentation, only the means for fungicide treatments averaged over varieties and sites are given in Table 14 along with a summary of the significance of the interactions. The analysis confirms that

there were strong site effects on the quantity of pathogen DNA detected at GS59 and significant site by fungicide interactions. However, the reductions in pathogen DNA measured at GS59 resulting from fungicide treatment were before appreciable visible symptoms of disease had developed. When averaged across sites and varieties, applications of Proline plus Comet at T1 and T2 reduced ramularia and rhynchosporium growth relative to untreated controls, but in each case the greatest reduction was observed with the combined T1 plus T2 application.

Detailed measurements of PAR interception and absolute leaf area were made in 2012, but not in 2011. Thus an analysis of the effects of fungicide timing on pre- and post-anthesis healthy area PAR interception is only possible for 2012. In a cross-site analysis there was no significant effect of fungicide treatment on PAR interception by healthy tissue pre-anthesis, nor any interaction between variety and fungicide application (Fig. 16) which is consistent with the small amount of visible disease on the crop before GS59. Fungicide treatment did increase ($P<0.001$) post-anthesis PAR interception with T2 applications (i.e. T2 and T1 + T2) resulting in significantly greater interception than controls or crops treated with just a T1 application.

Compared to T1 applications and untreated controls, the T2 and T1 plus T2 treatments significantly increased the number of grains produced per MJ of PAR interception by healthy tissue pre-anthesis (Fig. 17). Although the number of grains MJ^{-1} was greater following a T1 plus T2 application compared to T2 on its own, the difference was not statistically significant. There was no variety by fungicide interaction ($P=0.974$) on the number of grains MJ^{-1} and thus each variety responded in a similar way.

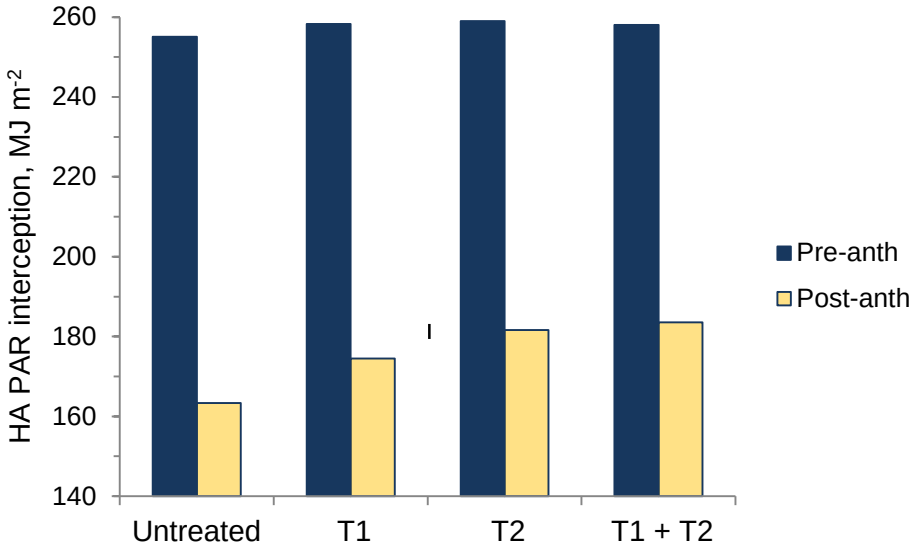


Fig. 16. Effects of fungicide (Proline plus Comet) timing on pre- and post-anthesis PAR interception by healthy area. Values are means across varieties from a cross-site analysis of experiments in 2012 where site was a random effect. There was no significant effect of fungicide on pre-anthesis PAR interception ($P=0.127$; V*F, $P=0.716$). Post-anthesis fungicide treatments differed significantly

(P<0.001, LSD (5%) 4.3; V*F P=0.113). Vertical bar shows the LSD for fungicide on post-anthesis interception.

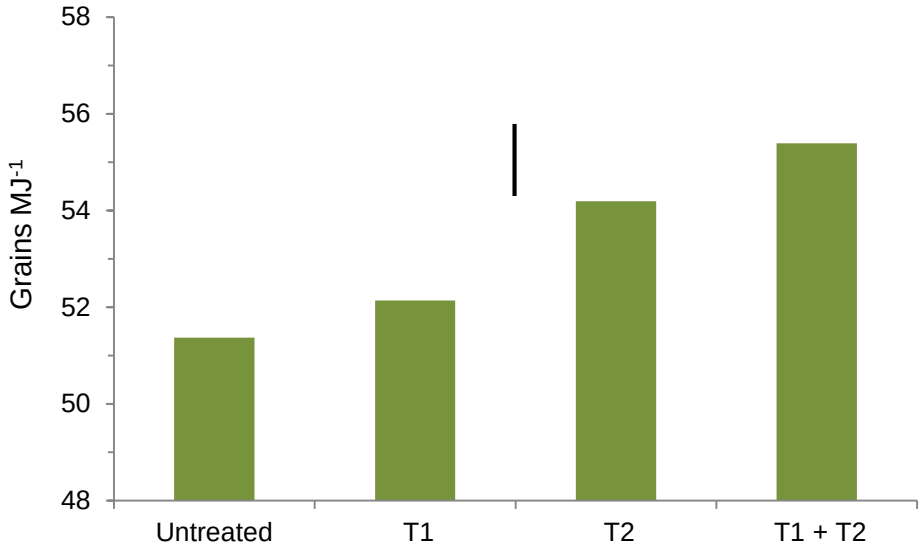


Fig. 17. Effects of fungicide (Proline plus Comet) timing on the number of grains produced per MJ of PAR intercepted by healthy tissue pre-anthesis. Values are means across varieties from a cross-site analysis of experiments in 2012 where site was included as a random effect; P<0.001 for fungicide treatment (V*F, P=0.974). Vertical bar shows the LSD (5%) for fungicide.

4.3.3. Economic benefit of treating resistant varieties

The economic benefit of the different fungicide timings has been estimated as the yield increase x the value of grain minus the cost of treatment. The latter is given as the cost of the fungicide plus an average cost of application. The values assumed in the analysis are based on typical prices in 2012 and are given in the footnote to Table 15.

Table 15. Economic margins from treating resistant varieties with Proline plus Comet at different timings.

	2012		2011		
Timing	Yield, t ha^{-1}	Margin £ ha^{-1}	Yield, t ha^{-1}	Margin £ ha^{-1}	Av margin £ ha^{-1}
T1	5.53	24.31	7.80	91.01	57.66
T2	5.84	95.61	7.78	85.83	90.72
T1 + T2	5.89	53.22	7.94	69.89	61.56
Untreated	5.20		7.18		

Footnote: based on spring barley malting price £230 t^{-1}, single timing fungicide cost £40.28 ha^{-1} (excluding application cost), application cost £12 ha^{-1}.

Averaged across the two years, which includes three sites in 2012 and two in 2011 with widely differing visible disease severities, the greatest economic benefit was achieved with a single application at T2. Although a T1 plus T2 application gave marginally greater yields, the yield improvement did not offset the extra cost of a second application. The margins achieved with a T1 application were on average lower than those with a T2, because the T1 gave a lower yield response in site-years where the disease pressure was high.

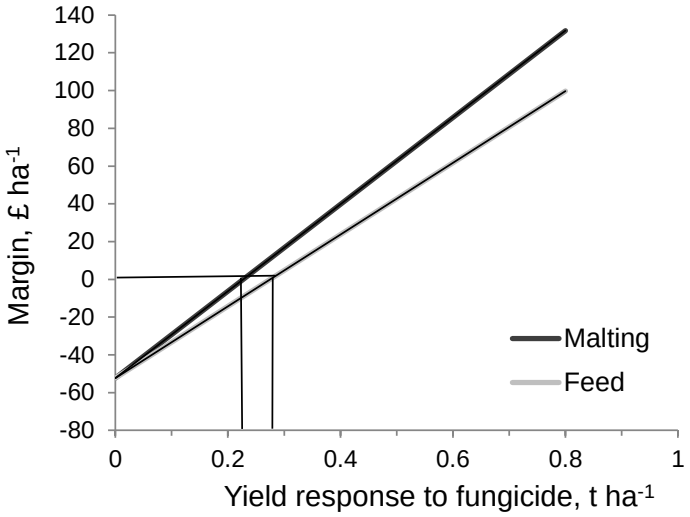


Fig. 18. Break-even yield responses to a single application of Proline plus Comet for spring barley crops grown for malting or feed based on a grain price for malting of £230 t^{-1} and feed price of £190 t^{-1}.

The yield response to fungicide required to break-even (i.e. where the cost of treatment equals the value of the additional grain produced) was 0.23 tha^{-1} for malting crops and 0.28 tha^{-1} for feed crops (Fig. 18). Yield responses exceeding this have been found in the all the current experiments (spanning 5 site-years) from single applications of fungicide, even at sites where there was little or no visible disease (e.g. SRUC 2011).

4.3.4. Conclusions

Results from section 4.2.3 demonstrated that the number of grains produced by cv. Westminster was increased by treatment with Proline plus Comet, but not Bravo. As these increases were not accompanied by increases in canopy green area or PAR interception by healthy area pre-anthesis, the number of grains produced per MJ^{-1} PAR intercepted was increased. Results from the current experiments show that this response was not confined to cv. Westminster, but was a more general response of spring barley. The same effect was observed across three varieties with no variety by fungicide interaction. Although treatment with Proline plus Comet reduced the growth of the main pathogens, ramularia and rhynchosporium by GS59 and before appreciable visible disease symptoms developed, this was not the cause of the increase in grain numbers. In section 4.2.3 it

was demonstrated that Bravo and Proline plus Comet were equally effective in reducing asymptomatic growth of ramularia and rhynchosporium, but only Proline plus Comet increased grain numbers.

A single application at T2 (GS45–49) was effective in eliciting the grain number response (Tables 10, 12; Fig 17), which suggests that the response occurred late during ear development, probably by increasing the survival of florets and hence the number of grains per ear. This is consistent with the general lack of effect of fungicide timing observed on the number of ears m^{-2}, the other sub-component of the grain number m^{-2} (Table 12). Moreover, the T2 application gave good protection of canopy green area (GLA) and post-anthesis healthy area PAR interception by these disease resistant varieties when disease pressure was high. There was little additional benefit of combining a T2 with an earlier T1 application in these experiments. Consequently, the T2 treatment gave the greatest financial margins of the different fungicide timings averaged over the five site-years.

4.4. Test new understanding across contrasting varieties and environments (objective 5). Industry partner contribution

4.4.1. Yields and yield response to fungicide

In general, disease severities were low for each of the experimental years 2009–2011. In 2009, half the sites recorded very low or no visible disease. Where visible disease was observed rhynchosporium, powdery mildew and ramularia were the main diseases. In spite of the low disease, when averaged across sites, a significant effect of fungicide on yield was found compared to untreated controls (Table 16), but little difference was detected between the different fungicide products and combinations. Thus Proline, Comet and a combination of Proline plus Comet gave significant yield increases over untreated controls ranging from 0.33–0.48 t ha^{-1}. In 2010, yields were in general lower than in 2009 and overall disease severity was zero or very low. Averaged across sites there was a significant effect of fungicide treatment, with each treatment giving an increase relative to controls. However, the yield responses tended to be lower than in 2009, and the combination of Proline and Comet was significantly more effective than either product used on its own. In 2011 significant yield responses were again found to each of the fungicide products and product combinations, compared to controls. Here the response to Proline and Proline plus Comet was significantly greater than that to Comet applied on its own. Averaged across the three sites, yield responses to Proline and Proline plus Comet were around 68% greater than those to Comet alone (0.37 and 0.22 t ha^{-1} respectively). Furthermore, the average response to Proline plus Comet tended to be more consistent between years than the response to either of the products used separately.

Table 16. Yield and yield responses to fungicide treatment, industry partner spring barley sites 2009–2011. Values are means for fungicide treatments across sites

		2009	2010	2011	All
Yield t ha^{-1} @85% DM	Untreated	6.26	5.03	5.42	5.51
	Comet	6.59	5.22	5.60	5.73
	Proline	6.74	5.19	5.90	5.88
	Proline + Comet	6.60	5.39	5.84	5.88
			.		
Yield response t ha^{-1}	Comet	0.33	0.19	0.18	0.22
	Proline	0.48	0.16	0.48	0.37
	Proline + Comet	0.34	0.36	0.42	0.37
Fung (F)		<0.001	<0.001	<0.001	<0.001
Site (S)		<0.001	<0.001	<0.001	<0.001
F*S		0.445	<0.001	0.002	<0.001
LSD (5%) F		0.19	0.15	0.17	0.10
(df)		(79)	(102)	(136)	(317)

Number of sites: 2009, 9; 2010, 10; 2011, 12

The yield increase in response to fungicide treatment was largely the result of an increase in the number of grains m^{-2}. There was no overall significant effect of fungicide on MGW, the number of ears m^{-2} or specific weight (Table 17). Yields differed markedly between sites and years and a large proportion of this could be explained by variation in the number of grains m^{-2} (R^2 = 0.92; Fig. 19).

Table 17. P values for fungicide treatment and site effects on yield and yield components. Data from different years have been pooled.

	Yield	MGW	Specific Wt	Ears m^{-2}	Grains m^{-2}
Fung (F)	<0.001	0.148	0.163	0.257	0.005
Site (S)	<0.001	<0.001	<0.001	<0.001	<0.001
F*S	<0.001	0.058	0.898	0.604	0.018

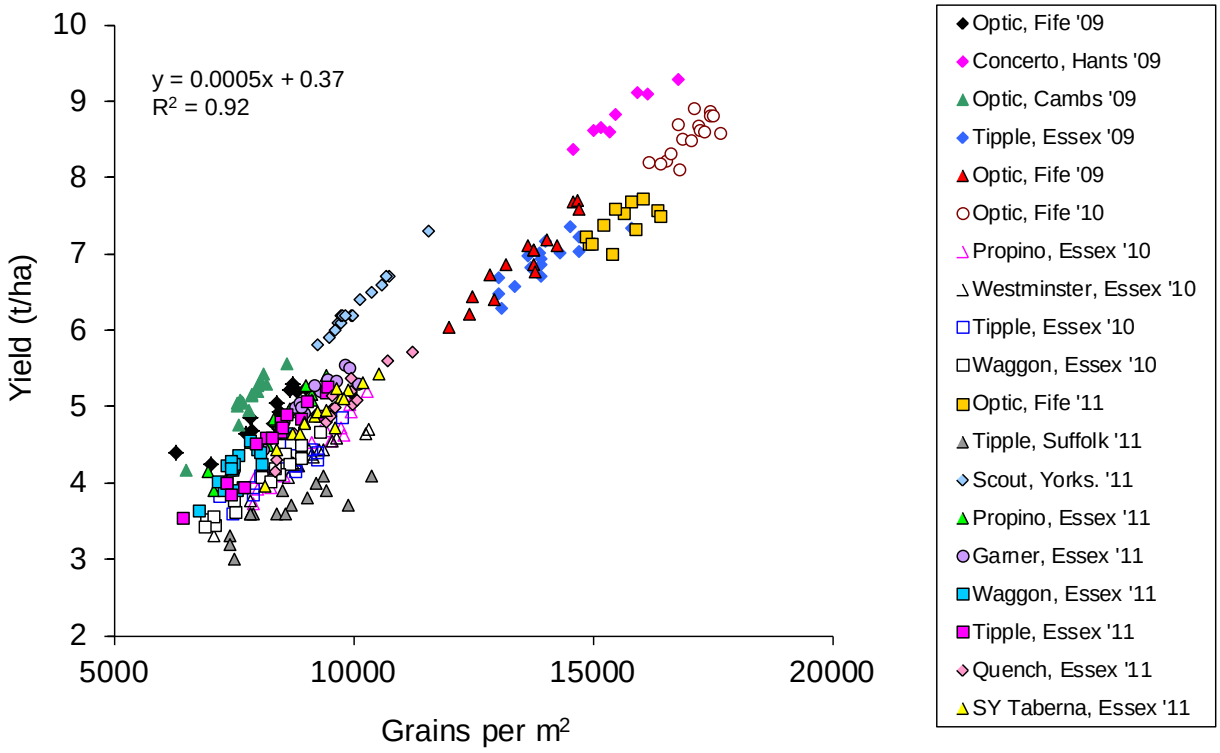


Fig.19. Relationship between yield and the number of grains m^{-2} for spring barley crops from different sites, years and fungicide treatments. Industry partner sites 2009–2011.

Figure 20 illustrates a linear relationship between the yield response to treatment with Proline plus Comet and the disease severity on untreated plots recorded during grain filling. However, the yield response to fungicide observed across sites and years was highly variable and only a small proportion of this variability was explained by the disease severity observed (R^2 = 0.27). The linear relationship is dependent on just one outlier. In the absence of disease, the yield response ranged from -0.35 to 1.35 t ha^{-1} with an average of 0.32.

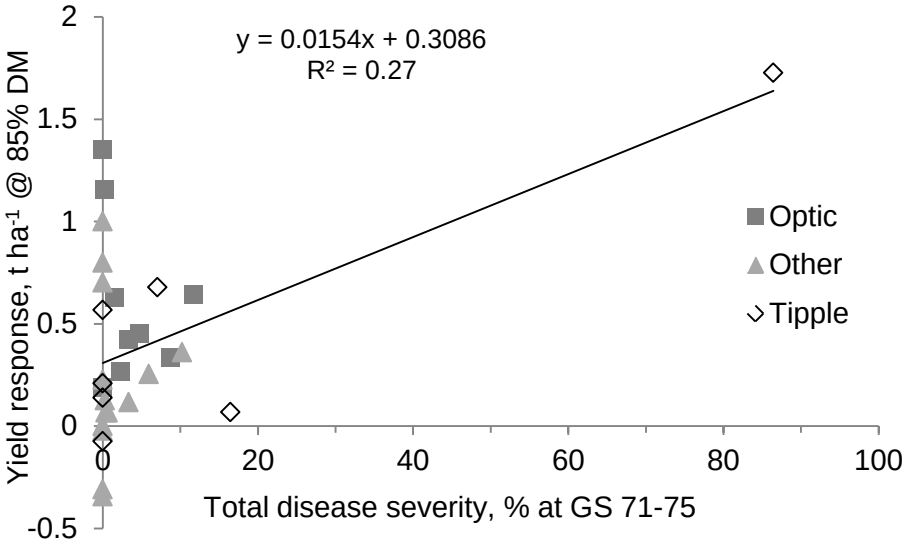


Fig. 20. Relationship between yield response to treatment with Proline plus Comet and total disease severity assessed at grain filling (around GS75). Line fitted by linear regression to data for all varieties combined. Industry partner sites 2009–2011.

The variation between sites was not simply the result of different varieties being grown. Optic and Tipple were common to several sites and showed the same lack of relationship between yield response and disease severity when plotted separately from the rest of the data (Fig. 20). There was also a large amount of variation in yield response for these varieties in the absence of disease, suggesting that it is site factors other than choice of variety that are responsible.

The experimental years 20092011 were relatively dry years at many of the sites and some crops were considered to be water stressed for part of the season and hence lower yielding. In order to test whether water stress may have altered the yield response to fungicide, the irrigation model 'Irriguide' (Silgram *et al.,* 2007) was used to estimate the potential soil moisture deficit throughout the growing season based on meteorological data, soil texture and assumed soil and rooting depth. The incidence and scale of estimated water stress for each site are shown in Fig 21. Water stress was predicted at some sites during the stem extension phase (between T1 and T2 fungicide timings). For a larger number of sites water stress was predicted for the later phase from flag leaf emerged (T2) to harvest. Out of 22 locations, nine had no stress, eight had >40% days stressed, and one site had severe stress just prior to harvest.

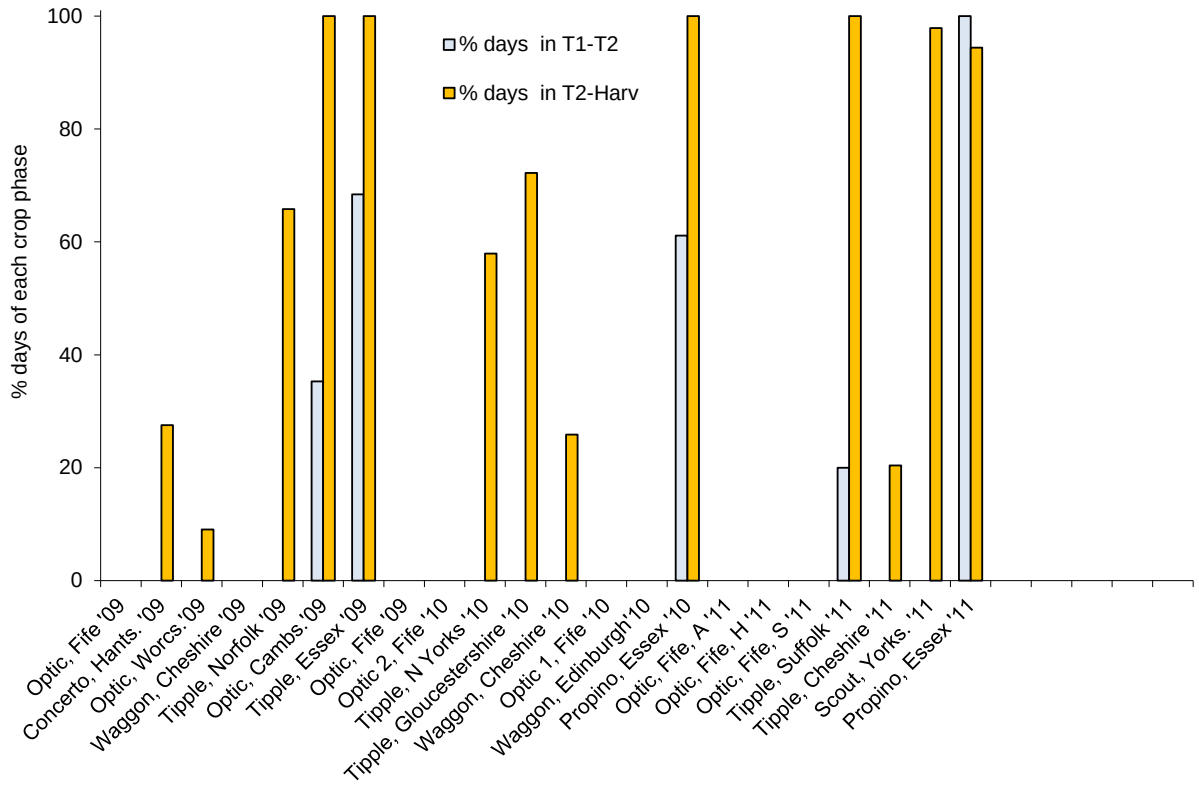


Fig. 21.Estimated incidence of water stress expressed in % of days SMD exceed the easily available water capacity of the root zone between T1 and T2 fungicide application dates and T2 to harvest.

The yield response to application of Proline plus Comet in the near absence of visible disease (disease severity <1% at GS75) was plotted against the duration of water stress predicted for the site (expressed as the % of days of the growing season with water stress) (Fig. 22). There was a weak negative relationship close to significance (P = 0.073) suggesting that water stress may have reduced the response to fungicide in the absence of disease to a small extent. However, as water stress explained relatively little of the total variation in yield response (R^2 = 0.23), other as yet unknown site factors must have had an overriding effect on the scale of the yield response.

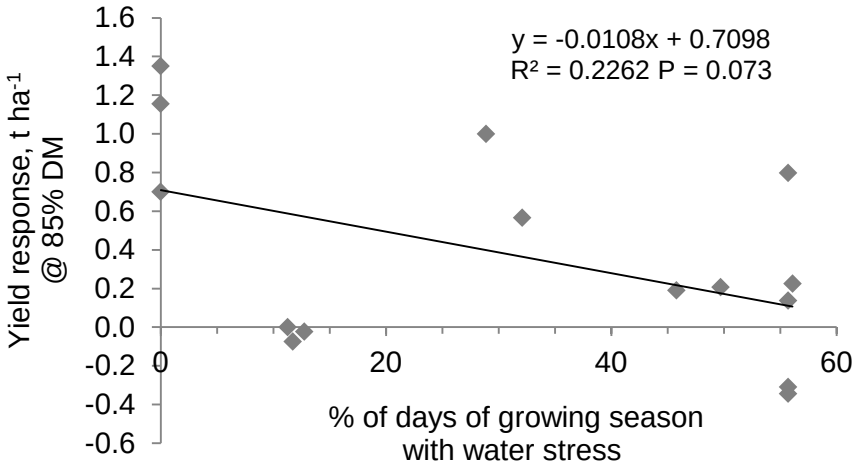


Fig. 22. Relationship between yield response to treatment with Proline plus Comet and the predicted water stress for sites and varieties with negligible visible disease (<1% disease severity at GS75).

4.4.2. Conclusions

The results from field experiments conducted by industry partners over a wide range of sites in the UK support those from the core research sites and demonstrate that triazole and strobilurin fungicides (Proline and Comet) can increase the yield of spring barley in the absence of visible disease. As at the core sites, the yield responses were found with both Proline (triazole) and Comet (strobilurin) and the combination of Proline plus Comet, although the responses on average were greater with Proline and Proline plus Comet than with Comet on its own. The yield responses were associated with a significant increase in grain numbers m^{-2} rather than an increase in MGW. This too is consistent with the findings from the core research sites. At ADAS and SRUC Proline and Comet increased grain numbers m^{-2}, a response that was attributed to direct effects of these fungicide groups on plant metabolism. Although increases in MGW were also observed following treatments with Proline and Comet at the core sites in 2012, this additional effect appeared to be associated with the control of visible disease and equivalent increases were found after treatment with Bravo. Thus, we can be confident that the conclusions reached from research relating to the response of spring barley to fungicides and the underlying mechanisms involved have general validity for spring barley production in the UK.

The average yield response observed to treatment with Proline plus Comet in the absence of disease at the industry sites was 0.32 t ha^{-1}. At the core research sites the average yield response for cv. Westminster was 0.4 t ha^{-1}. These averages mask considerable variation in the scale of the response at both industry and core sites. It is not clear what the cause of the variation is. There was little evidence to suggest that the yield response was *strongly* associated with the likely

duration of water stress experienced by the crop. Nor was the scale of the response related to the actual yield (data not shown), which suggests that nutritional deficiencies that can restrict yield are unlikely to be a major cause of variation in the response. The variation could be related to factors such as local weather conditions at the time of application influencing uptake and possible metabolism of the fungicide in plant tissues.

4.5. Impact of improved disease control on resource use efficiency and greenhouse gas costs of production (objective 6)

4.5.1. N use efficiency

Effects of fungicide treatments on elements of N use efficiency and the greenhouse gas costs of production were analysed using the fungicide product and timing data from experiments in 2012. The contrasting fungicide chemistries had differing effects on N uptake and allocation to the grain. The triazole and stobilurin fungicides (Proline and Comet) when used singularly had no significant effect on grain N concentration relative to untreated controls (Table 18). When used in combination there was a small, but significant reduction. Bravo by contrast gave a larger reduction in grain N concentration relative to controls. With Bravo the reduction resulted from a dilution of N in the grain from a greater yield following treatment. This is evident from the lack of effect of Bravo on total grain N offtake. By contrast, treatment with Proline and Comet, both alone and in combination, increased grain N offtake compared to Bravo treated crops and controls. As the increase in grain N offtake was accompanied by a broadly equivalent increase in grain yield, the N concentration changed little relative to controls. N use efficiency (NUE) can be defined in a number of ways, but it is often taken to be the grain yield per unit of N supply from soil plus fertiliser (Moll *et al*., 1982; Bingham *et al*. 2012b). The agronomic efficiency (Ae) is defined as the increase in yield of crops given N fertiliser relative to the yield of those with no N fertiliser, per unit of fertiliser applied (Ladha *et al*., 2005). As non-fertilised plots were not included in the current experiments, and measures of soil N supply were not available for all sites, standard definitions of Ae and NUE cannot be used. Here we define the NUE simply as the grain yield per unit of N fertiliser applied.

In principle, an increase in grain N offtake could arise from greater N uptake or greater remobilisation of N from leaves and stem and translocation to the grain. At the SRUC sites, pre-harvest samples were collected for determination of straw as well as grain N concentrations and biomass. This allowed a more detailed analysis of N partitioning and N use efficiency to be conducted.

Table 18. Effects of contrasting fungicide chemistry on grain N concentration, grain N offtake and NUE defined here as the grain yield per unit of fertiliser N applied. N % and NUE are calculated on the basis of 100% DM. Values are fungicide treatment means for cv. Westminster from a cross-site analysis of experiments in 2012. Site was analysed as a random effect and fungicide product as a fixed effect.

	Yield, t ha^{-1} @85% DM	N %	Grain N_{off}, kg ha^{-1}	NUE, kg grain (kg fert N)$^{-1}$
Untreated	5.25	1.80	80.36	37.75
Bravo	5.61	1.72	82.30	40.22
Comet	5.72	1.78	86.79	41.13
Proline	5.74	1.78	87.19	41.27
Proline + Comet	5.88	1.75	88.05	42.27
P	<0.001	0.008	<0.001	<0.001
LSD (5%)	0.208	0.045	3.972	1.482

Table 19. Effects of contrasting fungicide chemistry on partitioning of dry matter and N at harvest. Values are means for fungicide treatments across two sites (SRUC Edinburgh and Aberdeen). Site was analysed as a random effect and fungicide treatment as a fixed effect.

	Fungicide product						
	Untreated	Bravo	Comet	Proline	Proline + Comet	P	LSD (5%)
Grain yield, t ha^{-1} @100% DM	4.27	4.53	4.66	4.74	4.78	<0.001	0.172
Straw biomass, t ha^{-1}	5.24	5.58	6.14	5.91	6.05	0.004	0.490
Total biomass, t ha^{-1}	9.51	10.11	10.80	10.65	10.83	<0.001	0.546
HI	0.449	0.448	0.433	0.444	0.441	0.532	ns
RUE, g DM MJ^{-1} PAR	2.50	2.58	2.69	2.70	2.74	0.034	0.168
Straw N%	0.64	0.63	0.60	0.60	0.58	0.428	ns
Grain N%	1.80	1.72	1.78	1.80	1.74	0.038	0.060
Straw N_{off}, kg ha^{-1}	33.8	35.5	37.2	36.0	36.0	0.806	ns
Grain N_{off}, kg ha^{-1}	77.0	78.5	83.8	85.5	83.8	<0.001	3.48
Total N_{off}, kg ha^{-1}	110.7	113.9	121.0	121.4	119.8	0.019	7.40
NHI	0.695	0.689	0.693	0.704	0.699	0.808	ns
NutE grain, kg grain kg N_{off}^{-1}	39.75	41.63	39.95	40.21	41.44	0.229	ns
NutE biomass, kg biomass kg N_{off}^{-1}	89.22	93.94	93.05	91.35	94.51	0.147	ns
N_{off}:Fert applied	0.995	1.019	1.088	1.091	1.076	0.012	0.064

All fungicide treatments increased straw biomass along with grain yield compared to untreated controls, although the increase in straw biomass was statistically significant only for Proline and

Comet (Table 19). As a result there was no change in the dry matter harvest index (HI; grain yield / total biomass). The increase in grain N offtake observed with Proline and Comet in the three site analysis (Table 18) was also observed in the separate analysis of the SRUC sites (Table 19). Here the increase was associated with a comparable increase in total N offtake, such that fungicide treatment had no significant effect on the N harvest index (grain N_{off} / total N_{off}). These data imply that the increase in grain N offtake with Proline and Comet, but not Bravo, was largely the result of an increase in N uptake, rather the amount remobilised from straw. As such, the N offtake per unit of fertiliser applied (a measure of the N uptake efficiency) increased by 8–9% after treatment with Proline and Comet compared to Bravo and untreated controls. There was no significant effect of any fungicide treatment on the N utilisation efficiency (NutE), the efficiency with which the absorbed N was used to produce grain or total biomass, although there was a tendency towards greater (4–6%) values with Bravo and Proline plus Comet treatments.

Timing of applications of Proline and Comet had no significant effect on grain N concentrations when averaged across varieties (Table 20). Concentrations differed between varieties, but the fungicide timing by variety interaction was not significant (P>0.05). As yield was increased by all fungicide timings in these experiments (with the greatest increases being found with the T2 and the T1 + T2 treatments), grain N offtake and NUE were also increased in the same way.

Table 20. Effects of timing of applications of Proline plus Comet on grain N concentration, grain N offtake and NUE defined here as the grain yield per unit of fertilizer N applied. N % and NUE are calculated on the basis of 100% DM. Values are fungicide treatment means across three varieties from a cross-site analysis (three sites) of experiments in 2012. Site was analysed as a random effect with fungicide timing and variety as fixed effects.

	Yield, t ha^{-1} @85% DM	N %	Grain N_{off}, kg ha^{-1}	NUE, kg grain (kg fert N)$^{-1}$
Untreated	5.21	1.81	80.52	37.37
T1	5.53	1.80	85.07	39.66
T2	5.84	1.80	89.69	41.77
T1 + T2	5.87	1.78	89.22	42.06
V	0.261	0.002	0.225	0.226
F	<0.001	0.144	<0.001	<0.001
V*F	0.82	0.071	0.145	0.789
F LSD (5%)	0.1382	ns	2.546	0.959

The increase in grain N offtake observed with fungicide treatment in the 3 site analysis was also found when the SRUC sites were analysed separately (Table 21). The increase was the result of a

greater total N uptake (total N offtake and N_{off}:fert applied) especially with the T2 and T1 + T2 treatments. In addition, there was some evidence that the T2 treatments may have increased N partitioning to the grain as there was a significant increase in NHI and decrease in straw N%. Late fungicide applications also resulted in small (4%) but significant improvements in NutE grain and NutE biomass. There was no significant interaction between variety and fungicide for any of the N use characteristics, indicating that each variety responded to fungicide treatment in a comparable way.

4.5.2. Greenhouse gas (GHG) costs of production

Greenhouse gas costs of production are expressed as CO_2 equivalents per tonne of grain yield produced. Fungicide treatment led to a significant decrease in GHG costs of 3 – 9% depending on the fungicide product applied (Fig. 23). These differences between products largely reflect differences in the scale of the yield response to the contrasting fungicide chemistries, with the responses being smaller for Bravo compared with Proline and Comet. There was a slightly greater CO_2e cost associated with the use of Bravo as the treatment involved a larger mass of active ingredient. The greatest reduction in emissions came from the use of Proline plus Comet. Timing of Proline plus Comet also had a significant impact on GHG costs with T2 and T1 plus T2 reducing emissions per tonne of grain by 9% relative to untreated crops, compared to 5% for the T1 application on its own.

4.5.3. Conclusions

In summary, the analysis of N use characteristics has demonstrated that Proline and Comet can increase N uptake by the crop compared to untreated crops or those treated with Bravo, and can lead to marginal increases in N partitioning to the grain. Late applications during booting were more effective in eliciting this response than an application at the start of stem extension. Strobilurin fungicides have been reported to increase N uptake and reduce soil mineral N concentrations of wheat at harvest (Bryson, 2000; Dimmock and Gooding, 2002). In experiments in which canopy lifespan of wheat was varied using a fungicide programme of different mixtures of epoxiconazole, azoxystrobin and picoxystrobin, grain N offtake was increased in the range of 10–45 kg N ha^{-1} and NHI by 0.02–0.10 relative to untreated controls depending on the year (Ruske *et al.,* 2003). In these experiments the increases were linearly related to improvements in post-anthesis disease control and canopy lifespan and it was concluded that the fungicide effects were mediated through the increased leaf area duration and not independently through previously reported effects of strobilurins on nitrate reductase activity (Glaab and Kaiser, 1999; Dimmock and Gooding, 2002; Ruske et al., 2003). Moreover, the increases in N uptake and grain N yield were accompanied by increases in total above ground dry matter and dry matter harvest index (Ruske *et al*., 2003).

Table 21. Effects of timing of applications of Proline plus Comet on partitioning of dry matter and N at harvest. Values are means for fungicide treatments across three varieties and two sites (SRUC Edinburgh and Aberdeen). Site was analysed as a random effect with variety and fungicide treatment as a fixed effects. P values are given for variety (V), fungicide (F) and the V*F interaction.

	Fungicide timing				P value			
	Untreated	T1	T2	T1 + T2	V	F	V*F	LSD (5%)
Grain yield, t ha^{-1} @100% DM	4.20	4.45	4.65	4.71	0.444	<0.001	0.731	0.132
Straw biomass, t ha^{-1}	5.32	5.76	5.72	5.99	0.032	<0.001	0.228	0.302
Total biomass, t ha^{-1}	9.51	10.21	10.37	10.70	0.049	<0.001	0.435	0.391
HI	0.442	0.437	0.449	0.441	0.010	0.156	0.122	ns
Straw N%	0.63	0.58	0.57	0.57	0.963	0.003	0.642	0.035
Grain N%	1.82	1.81	1.80	1.78	0.050	0.278	0.149	ns
Straw N_{off}, kg ha^{-1}	34.4	33.9	33.1	35.6	0.369	0.502	0.358	ns
Grain N_{off}, kg ha^{-1}	76.6	81.2	84.5	84.5	0.479	<0.001	0.218	2.83
Total N_{off}, kg ha^{-1}	111.0	115.1	117.6	120.1	0.711	0.006	0.363	5.12
NHI	0.704	0.714	0.730	0.719	0.020	0.014	0.199	0.016
NutE grain, kg grain kg N_{off}^{-1}	39.3	39.77	40.92	41.02	0.686	0.032	0.419	1.36
NutE biomass, kg biomass kg N_{off}^{-1}	89.2	91.9	91.8	93.5	0.068	0.024	0.136	2.76
N_{off}:Fert applied	1.00	1.03	1.05	1.08	0.742	0.007	0.379	0.045

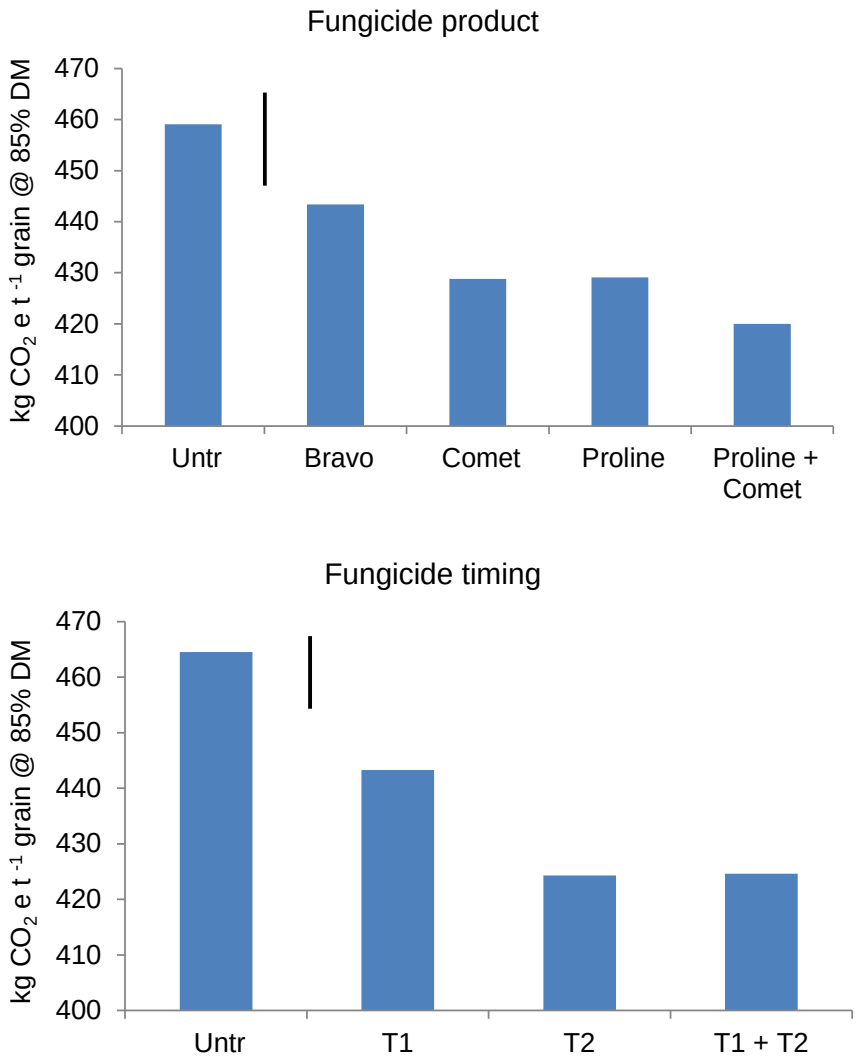


Fig. 23. Effects of fungicide product and timing on greenhouse gas costs of producing a tonne of spring barley grain. Values are fungicide treatment means from a cross site analysis of experiments in 2012. Fungicide products were applied at T1 and T2 to cv. Westminster. Fungicide timing was for Proline plus Comet applied to 3 varieties; as there was no significant variety*fungicide interaction (P=0.733) values presented are for fungicide effects averaged across varieties. Vertical bars are the LSDs (5%) for fungicide.

In the current study on lower yielding spring barley crops, the increases in N uptake and NHI with triazole and strobilurin applications were smaller than those reported for wheat (Ruske *et al.,* 2003) and were not associated with increases in dry matter harvest index. More importantly, our data suggest that the effects of the triazole (Proline) and strobilurin (Comet) on N uptake and allocation to grain were *not* a direct consequence of the increased green canopy duration, because applications of Bravo resulted in identical improvements % GLA and post-anthesis healthy area PAR interception but no increase in N uptake or grain N offtake.

The manufacture and application of pesticides accounts for only a small percentage of the total GHG costs of production (Berry *et al*., 2008). Consequently, increases in yield resulting from the control of disease, or more direct physiological effects of fungicides, are associated with a reduction in CO_2 equivalent emissions per tonne of grain produced. In an analysis of fungicide trials involving over 800 variety/site/season combinations, average yields of UK grown winter wheat were increased from 8.42 to 10.20 t ha^{-1} following fungicide treatment, reducing emissions from 386 to 327 kg CO_2e t^{-1} grain (Berry *et al*., 2008). As CO_2e emissions t^{-1} grain are non-linearly related to actual yield (proportionately larger emissions t^{-1} grain occurring at lower yield), the estimated benefits of fungicide treatment on spring barley are in line with those reported for winter wheat. Thus, emissions were reduced from 460 to 420 kg CO_2 e t^{-1} grain for a yield increase from 5.2 to 5.9 t ha^{-1}. It is worth emphasising that for a comparable level of disease control, Proline plus Comet reduced emissions to a greater extent than Bravo. Although the analysis was conducted on data from experiments in 2012 where appreciable foliar disease developed, we can estimate that a 5% reduction in emissions would have occurred for a typical yield increase of 0.4 t ha^{-1} in response to Proline plus Comet in the absence of visible disease.

5. Key messages & practical implications

Overview

This project has provided new insights into the physiological responses of spring barley crops to fungicide treatments under varying disease pressure. The improved understanding will enable current disease management practices to be evaluated against objective physiological criteria that were not available hitherto. The requirement for fungicide treatment can then be assessed from a consideration not only of the efficacy of disease control, but also the likely yield response and environmental footprint of the crop, accounting for direct effects of fungicides on plant metabolism, the crop's resource use efficiency and the greenhouse gas costs of production. Specifically, the project has provided, for the first time, an estimate of the duration of post-anthesis canopy protection required by UK grown spring barley in order to maximise grain yield. This provides a benchmark against which the effects of varying fungicide rates, application timings and active ingredients on post-anthesis canopy lifespan can be assessed. The project has also provided new understanding of the mechanisms by which prothioconazole and pyraclostrobin (a triazole-strobilurin fungicide programme) can increase the yield of barley compared with other fungicide chemistries (specifically chlorothalonil). This will be useful for designing and justifying crop management strategies for use with disease resistant varieties and crops at low disease risk sites.

Key scientific messages

The key scientific messages with implications for crop management are summarised below:

- Under UK conditions, canopy light (PAR) interception of spring barley crops requires protection for approximately the first 75% of grain filling. This equates to a period of around 3–5 weeks from 50% ear emergence depending on the site and season.
- In low disease pressure situations, a T1 fungicide applied at the start of stem extension GS30–31 can provide sufficient protection post-anthesis over this period to maximise grain yield. Under higher disease pressure, an additional application at T2 is required; a T2 applied during booting gives longer duration protection post-anthesis than one applied earlier at GS37.
- Applications after flowering can prolong canopy duration and light interception even further, but do not increase yield as the extra canopy duration afforded occurs late in, or after, the critical period requiring protection. Currently, opportunities for using fungicides after flowering in barley are limited because of product label restrictions, although there is interest amongst growers in the possibility of using fungicides after ear emergence to protect against head diseases. There was no evidence from the current study to support the need to apply fungicides after flowering to maximise yield, even in site-years when post-anthesis disease (e.g. ramularia) epidemics were severe and the risk of fusarium infection high (e.g. SRUC 2012). Whilst late applications after ear emergence increased post-anthesis PAR interception, they had little effect on the date of final canopy (stem) senescence, and hence posed little risk to harvesting.
- A triazole-strobilurin fungicide programme (specifically prothioconazole and pyraclostrobin) can increase yield even when there is little or no visible disease present. Yield increases in the absence of disease were in the order of 0.3–0.4 t ha^{-1}.
- A comparison of the response to prothioconazole (Proline) and pyraclostrobin (Comet) with that to chlorothalonil (Bravo) has provided new insights into the mechanism by which the triazole-strobilurin fungicide programme elicits these yield responses. Bravo increased yield through an increase in MGW by controlling visible disease and increasing post-anthesis PAR interception. Proline plus Comet increased MGW and post-anthesis PAR interception to the same extent, but provided an additional yield increase through an increase in grain numbers. An accompanying increase in RUE ensured there was sufficient assimilate available to fill the additional grains. The effects of Proline and Comet on grain numbers and RUE occur when used separately or in combination. They cannot be ascribed to the control of visible disease, control of asymptomatic pathogen infection, control of leaf surface saprophytes, increase in leaf growth, or delayed leaf senescence. The evidence points to other direct effects of the fungicides on plant metabolism.
- The mechanisms underlying the yield response of barley identified in the current study differ markedly from those implicated in the yield response of wheat to triazoles and strobilurins. In wheat, yield increases are usually attributed to the delayed leaf senescence and extended post-anthesis canopy duration resulting from fungicide application.

- The increase in grain numbers following application of Proline and Comet has been found in the relative absence of disease over a wide range of sites and varieties.
- A single application of Proline plus Comet can elicit the grain number response, and an application at the T2 timing (GS45–49) was more effective than one at the start of stem extension (GS30–31).
- There was evidence that Bravo and Proline plus Comet can prolong post-anthesis green leaf area in the absence if visible disease. This might be related to the control of leaf surface saprophytes as some evidence was found of a reduction in fungal hyphae and the quantity of *Cladosporium* sp. DNA (a dominant saprophyte on barley) on the leaf surface following applications of Bravo, Proline and Comet. However, there is insufficient evidence from the current study to demonstrate whether the control of saprophytes contributed to the observed increase in MGW.
- By increasing yield relative to untreated crops, applications of fungicide reduced the greenhouse gas costs per tonne of grain produced. Reductions were greater with Proline and Comet than Bravo. Proline and Comet also increased N uptake and hence the N fertiliser use efficiency (yield per unit of N fertiliser applied), without significantly altering grain N concentrations. By contrast, Bravo reduced grain N concentration by increasing yield without an accompanying increase in N uptake and allocation to the grain.

Practical implications for disease management and fungicide stewardship

The findings summarised above support the following strategies for managing foliar disease in spring barley crops in contrasting disease-risk situations.

Low disease pressure sites and/or where resistant varieties are being grown

Whilst many varieties offer a high level of resistance to powdery mildew, resistance to other key pathogens including rhynchosporium and ramularia is not complete (HGCA, 2013b). It is important, therefore, to monitor disease even in low risk situations and treat the crop if disease appears. If no visible disease has developed by booting, a single application of fungicide at the T2 timing (GS45–49) will protect against late disease. Application of a triazole plus strobilurin is likely to give yield benefits, even if no late visible disease develops, by increasing grain number formation and improving radiation use efficiency. The treatment can be justified economically as the average yield response in the absence of disease exceeds that required to cover the cost of treatment. It also provides additional benefits in terms of reducing the greenhouse gas cost per tonne of grain production and increasing the efficiency of N fertiliser use. Chlorothalonil is unlikely to provide the same yield and environmental benefits even where equivalent protection from late season disease can be achieved. However, chlorothalonil may be the preferred option at sites where there is a risk of high grain N% in crops grown for the malting market as protection from late season disease is associated with a lower uptake of N compared to the triazoles and strobilurins.

Higher disease pressure sites and/or where susceptible varieties are being grown
The crop should be monitored for disease. Where early disease develops or where there is a high risk of it appearing, fungicide application at T0 (GS25) or T1 (GS30–31) may be required. A T2 application will be needed to protect the canopy against post-anthesis disease. The target is to provide protection for the first 75% of grain filling. A T2 application at booting (GS45–49) should give sufficiently long protection to maximise yield even under high disease pressure. Later applications to extend canopy lifespan are unnecessary. If the T0 or T1 application has been missed and disease is developing, an earlier T2 (at GS37) is a compromise that may give adequate protection during grain filling under moderate disease pressure. Use of a triazole and/or strobilurin at T2 would be expected to increase grain numbers and RUE even if disease development is lower than expected.

These strategies are based on the assumption that triazoles and strobilurins other than prothioconazole and pyraclostrobin are able to elicit increases in grain numbers and RUE independently of disease control. However, this assumption has still to be tested. Responsible fungicide stewardship requires that mixtures of active ingredients with contrasting modes of action are used to delay the development of fungicide insensitivity in pathogen populations. It is necessary, therefore, to investigate whether other fungicide chemistries, including the SDH inhibitors and other triazole and strobilurin active ingredients, are as effective in eliciting these responses in order to provide growers with the information needed for selecting and mixing suitable active ingredients. This and other recommendations for research are highlighted below.

Recommendations for research

- Determine which fungicide chemistries are able to increase grain numbers and RUE in the absence of disease, and evaluate the efficacy of the major fungicide active ingredients currently on the market. This will enable growers to design fungicide programmes that maximise yield via direct metabolic effects and the control of disease, whilst minimising the risk of fungicide insensitivity developing. This research should also include an investigation of which fungicides increase crop N uptake so that potential risks to grain quality can be evaluated alongside the expected benefits of enhanced yield and good disease control.
- The current study has demonstrated that grain numbers and RUE can be increased by direct effects of certain types of fungicides on plant metabolism. An understanding of the physiological, biochemical and molecular mechanisms underlying these responses could identify novel targets for plant breeders to enhance barley yields in the absence of disease without the application of chemicals. This would help rationalise the use of fungicides ensuring that they are used only where fungal pathogens threaten crop growth.

- Previous research in Denmark (Smedegaard-Petersen and Tolstrup, 1985) has suggested that leaf surface saprophytes can reduce barley yields in the absence of visible disease symptoms and has identified species of the genus *Cladosporium* as being amongst the most important in this respect. In the current study, some evidence was found of an increase in leaf lifespan after treatment with fungicide (in the absence of visible disease) that could be associated with the control of saprophytes. However, there were too few site-years without post-anthesis visible disease to establish whether the saprophytes themselves had a negative effect on the yield of barley in the UK. Thus it is not possible to conclude at this stage whether the control of non-pathogenic leaf fungi should be a target of crop management to increase yield, as is suggested by the Danish research. Research is needed, therefore, to establish: (i) whether the control of *Cladosporium* and other species of saprophytes increases leaf lifespan, (ii) whether such an increase in leaf lifespan in the absence of visible disease can increase yield under UK conditions and if an increase in yield is found and (iii) what methods (chemical and non-chemical) can be used to control saprophyte populations on barley leaves.

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8. Appendices

Appendix 1

Table A-1.1. Site and husbandry details ADAS

	ADAS 2009	ADAS 2011	ADAS 2012
Grid ref	SO55695 48193	SO40764 71259	SO32527 62815
Previous crop	Potatoes	Spring Barley	Spring Barley
Soil type	Silty Clay Loam	Silty Clay Loam	Silty Clay Loam
Soil analysis			
pH	6.9	6.3	6.5
P	Index 5 (78mg/l)	Index 3 (27.6mg/l)	Index 2 (17.4mg/l)
K	Index 3 (332mg/l)	Index 2+ (201mg/l)	Index 1 (120mg/l)
Mg	Index 3 (106mg/l)	Index 3 (133mg/l)	Index 3 (130mg/l)
Organic matter %	2.7%	3.6%	3.2%
Sowing date	13/03/2009	22/03/2011	15/3/2012
Fertilizer	07/04/2009: 100 kg/ha N	7.5 t/ha broiler manure applied to seed bed, approx 225 kg/ha N	28/9/2011 104 kg/ha P_2O_5, 104 kg/ha K_2O 6/4/2012 45 kg/ha N & 50 kg/ha SO_3 12/5/2012 93 kg/ha N
Herbicide, insecticide & trace elements	23/05/2009: Ally 48 g/ha + Starane 1.2 l/ha	22/03/2011: Defy 1.5 l/ha + Hurricane 0.1 l/ha 06/06/2011: Ally Max 0.03 kg/ha, Hatchet Xtra 0.75 l/ha, Curfew 0.25 l/ha	29/05/2012 Ally Max SX 30 g/ha, Hatchet 0.5 l/ha and Curfew 0.25 l/ha
Harvest date	20/08/2009	21/08/2011	08/09/2012

Table A.1.2. Site and husbandry details SRUC

	2009	2010	2011	2012	2012
Grid ref	NT244653	NT252658	NT256635	NT245649	NJ870113
Previous crop	Grass/clover	Spring barley	Spring barley	Spring barley	Spring barley
Soil type	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy silt loam
Soil analysis					
pH	5.5 (lime applied)	5.8 (lime applied)	5.8 (lime applied)	6.0	6.0 (estimated)
P	M	M	M	VH	No data
K	M	M	M	VH	No data
Mg	M	M	M	H	No data
Organic matter %	7.5	4.9	6.9	8.2	8.5 (estimated)
Sowing date	24/3/2009	16/3/2010	21/3/2011	15/3/2012	29/3/2012
Fertilizer, (kg/ha N:P_2O_5:K_2O)	24/3/2009: 40:40:40	22/3/2010: 60:60:60 19/4/2010: 50:0:0	28/3/2011: 60:60:60 11/4/2011: 70:0:0	19/4/2012: 60:60:60 13/4/2012: 40:0:0	28/3/2012: 20:50:85 10/5/2012: 70 kg/ha N + 40 kg/ha S
Herbicide, insecticide & trace elements	10/5/2012 Harmony MSX (100 g/ha) + Compitox plus (0.75 l/ha) + MetrolBiox (0.5 l/ha) + Mantrac 500 (l.0 l/ha); 10/9/2009 Reglone (3.0 l/ha)	4/5/2010 Harmony MSX (100 g/ha) + High load Mircam (0.75 l/ha) + Oxytril (0.25 l/ha); 26/4/2010 Calciprill (600 kg/ha); 29/4/2010 Mantrac (1.0 l/ha); 18/5/2101 Mantrac (1.0 l/ha)	29/4/2011 Harmony MSX (100 g/ha) + High load Mircam (0.5 l/ha) + Oxytril (0.5 l/ha); 15/4/2011 Mn (2.0 l/ha) & Calciprill (600 kg/ha); 29/4/2011 Mn (2.0 l/ha); 9/5/2011 Thio-S (5 l/ha)	3/5/2012: Mantrac 500 (1.0 l/ha) Headland sulphur (3.0 l/ha) 9/5/2012: Harmony MSX (73 g/ha) + Oxytril (0.4 l/ha) + Compitox Plus (0.2 l/ha) + High load Mircam (0.5 l/ha)	23/5/2012 Ally Max SX (30 g/ha) + Alpha Briotril (0.5 l/ha) + Optica (0.5 l/ha); 11/6/2012 $MnSO_4$ (4.0 kg/ha); 20/6/2012 Halmark (50 ml/ha)
Harvest date	17/9/2009	27/8/2010	5/9/2011	5/9/2012	18/9/2102

Appendix 2.

Table A-2.1. Details of industry partner sites

Variety	Year	Partner	County	Grid Ref	Soil type	Drilled	Seed rate/m^2	Prev crop	Date Fung 1	GS Fung 1	Date Fung 2	GS Fung 2	Date harvest	*RL Mil	*RL YR	*RL BR	*RL RH
Optic	2009	BASF	Fife	NO134 073	SL	26/03/09	400	SB	22/05/09	31	24/06/09	39-45	09/09/09	5.0	7.9	6.5	3.8
Concerto	2009	BASF	Hants.	SU472 412	SCL	16/03/09	350	SB	14/05/09	30	03/06/09	49-53	11/08/09	8.4	7.0	5.7	3.7
Optic	2009	Bayer	Worcs.	SO882768	SCL	29/03/09	375	SB	18/05/09	30	03/06/09	49	19/08/09	5.0	7.9	6.5	3.8
Waggon	2009	Bayer	Cheshire	SJ739781	SCL	20/03/09	375	WW	07/05/09	24	09/06/09	39	09/09/09	8.5	6.0	5.5	3.0
Tipple	2009	Bayer	Norfolk	TF710292	SiCL	15/01/09	210	BV	28/04/09	32	19/05/09	49	03/08/09	7.4	4.4	6.4	4.0
Optic	2009	Masstock	Cambs.	TL405473	SL	02/04/09	350	WOSR	12/06/09	52	29/06/09	76	01/09/09	5.0	7.9	6.5	3.8
Tipple	2009	Masstock	Essex	TL665245	SL	04/04/09	350	WO	12/06/09	41	01/07/09	71-73	01/09/09	7.4	4.4	6.4	4.0
Optic	2009	UAP	Fife	NO505007	SL	24/03/09	350	WW	01/06/09	32	13/06/09	39-41	13/09/09	5.0	7.9	6.5	3.8
Optic	2010	UAP	Fife	NO502005	SL	16/04/10	350	WW	25/05/10	31	11/06/10	45	21/08/10	5.0	7.9	6.5	3.8
Tipple	2010	Bayer	Yorks.	TA858552	SL	27/03/10	375	SB	19/05/10	25	05/06/10	39	01/09/10	7.4	4.4	6.4	4.0
Tipple	2010	Bayer	Gloucs.	SP087256	CL	06/03/10	375	WW	14/05/10	30	21/06/10	59	01/09/10	7.4	4.4	6.4	4.0
Waggon	2010	Bayer	Cheshire	SJ739781	SCL	22/04/10	375	ZM	04/06/10	30	05/07/10	39	01/09/10	8.5	6.0	5.5	3.0
Optic	2010	BASF	Fife	NT 318 999	SL	20/03/10	400	WO	26/05/10	30	14/06/10	39-45	22/08/10	5.0	7.9	6.5	3.8
Propino	2010	Masstock	Essex	TM028290	SL	23/03/10	350	SB	13/05/10	30	18/06/10	55	18/08/10	7.7	3.4	4.7	6.7
Westminster	2010	Masstock	Essex	TM028290	SL	23/03/10	350	SB	13/05/10	30	18/06/10	55	18/08/10	8.8	6.9	5.8	7.6
Tipple	2010	Masstock	Essex	TM028290	SL	23/03/10	350	SB	13/05/10	30	18/06/10	55	18/08/10	7.4	4.4	6.4	4.0
Waggon	2010	Masstock	Essex	TM028290	SL	23/03/10	350	SB	13/05/10	30	18/06/10	55	18/08/10	8.5	6.0	5.5	3.0
Waggon	2010	BASF	Fife	NT256653	SL	05/05/10	360	SB	11/06/10	30	29/06/10	45-59	15/09/10	8.5	6.0	5.5	3.0
Optic	2011	UAP	Fife	NO507009	SL	12/04/11	350	WW	06/06/11	31	20/06/11	45	31/08/11	5.0	7.9	6.5	3.8
Optic	2011	BASF	Fife	NT247649	SL	21/03/11	360	SB	13/05/11	31	10/06/11	45-49	30/08/11	5.0	7.9	6.5	3.8
Optic	2011	BASF	Fife	NT299990	SL	26/03/11	400	WW	09/05/11	30	10/06/11	39-45	23/08/11	5.0	7.9	6.5	3.8
Tipple	2011	Bayer	Cheshire	SJ739781	SCL	11/04/11	375	WOSR	10/05/11	30	14/06/11	53	02/09/11	7.4	4.4	6.4	4.0
Tipple	2011	Bayer	Suffolk	TM123761	SL	10/03/11	375	WW	04/05/11	30	17/05/11	39	18/08/11	7.4	4.4	6.4	4.0
Scout	2011	Bayer	Yorks.	TA858552	SL	23/03/11	375	SB	10/05/11	31	31/05/11	45	02/09/11	8.0	2.0	6.0	4.0
Propino	2011	Masstock	Essex	TM021272	SL	21/03/11	375	SB	12/05/11	32	14/06/11	57-58	29/09/11	7.7	3.4	4.7	6.7
Garner	2011	Masstock	Essex	TM021272	SL	21/03/11	375	SB	12/05/11	32	14/06/11	57-58	29/09/11	8.5	6.5	4.7	7.0
Quench	2011	Masstock	Essex	TM021272	SL	21/03/11	375	SB	12/05/11	32	14/06/11	57-58	29/09/11	8.6	4.5	3.7	7.6
Taberna	2011	Masstock	Essex	TM021272	SL	21/03/11	375	SB	12/05/11	32	14/06/11	57-58	29/09/11	8.0	*	*	6.0
Tipple	2011	Masstock	Essex	TM021272	SL	21/03/11	375	SB	12/05/11	32	14/06/11	57-58	29/09/11	7.4	4.4	6.4	4.0
Waggon	2011	Masstock	Essex	TM021272	SL	21/03/11	375	SB	12/05/11	32	14/06/11	57-58	29/09/11	8.5	6.0	5.5	3.0

RL = HGCA recommended list ratings for disease resistance; 9 = high resistance, 1 = low. Mil = powdery mildew, YR = yellow rust, BR = brown rust, RH = rhynchosporium. Si = silt, C = clay, L = loam. WW = winter wheat, SB = spring barley, ZM = maize, BV = sugar beet, WO = winter oats, WOSR = winter oilseed rape.

Appendix 3a. Meteorological conditions in shaded and non-shaded plots post-anthesis

Table A-3.1. Atmospheric conditions

Site	Year	Measurement	Non-shaded	Shaded
SRUC	2010	Mean air temp, ^{o}C	13.5	13.6
		Mean RH, %	82.7	84.7
		Mean daily rainfall, mm	3.5	4.3
SRUC	2011	Mean air temp, ^{o}C	12.0	12.1
		Mean RH, %	83.1	84.6
		Mean daily rainfall, mm	1.8	1.5

Note: Equipment failure in 2011 restricted the measurement period to the final part of grain filling (18 Aug to 4 Sept).

Table A-3.2. Gravimetric soil moisture content (g H_2O g^{-1} DW soil) at the end of the post-anthesis shading period

Site	Year	Soil depth, cm	Early shading	Mid shading	Non-shaded
SRUC	2010	0-30	0.224	0.225	0.214
		30-60	0.204	0.200	0.188
		60-90	0.188	0.216	0.168
	p values	Shading		0.501	
		Depth		0.159	
		Shading*Depth		0.693	
SRUC	2011	0-30	0.232	0.251	0.233
		30-60	0.213	0.217	0.202
		60-90	0.178	0.180	0.177
	p values	Shading		0.868	
		Depth		0.021	
		Shading*Depth		0.940	

Appendix 3b. Meteorological conditions during pre-anthesis shading ADAS 2009

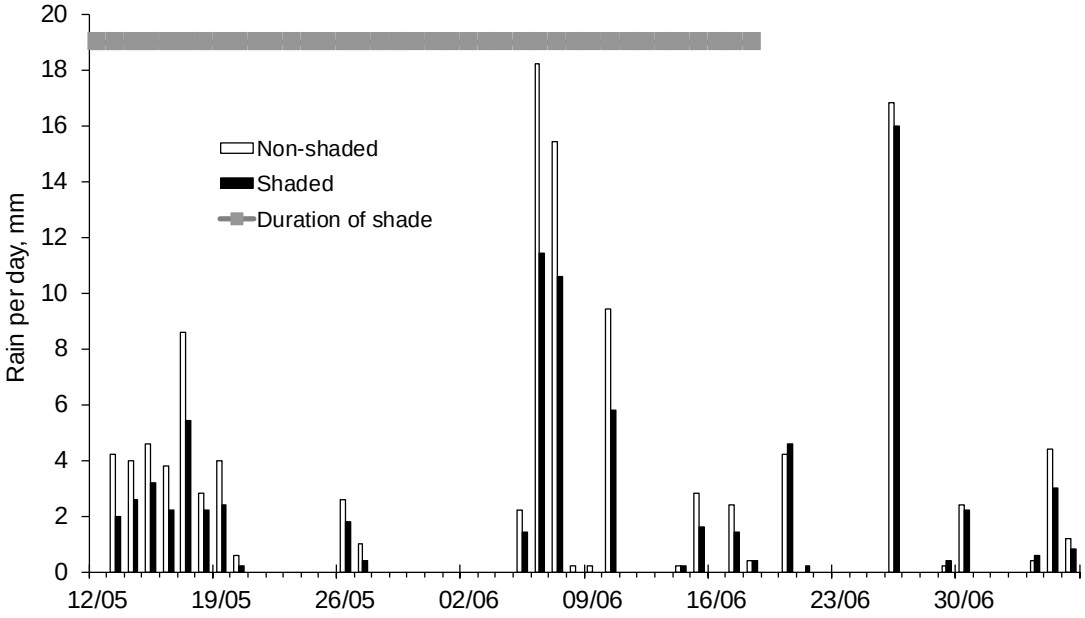

Fig. A-3.1. Rainfall collected in open (non-shaded) and shaded plots both during pre-anthesis shading and after shade removal. Shade netting appeared to reduce some transmission of rain, although this may in part be the result of pooling of water on the nets and transmission away from the rain gauge (cf. Table A-3.1).

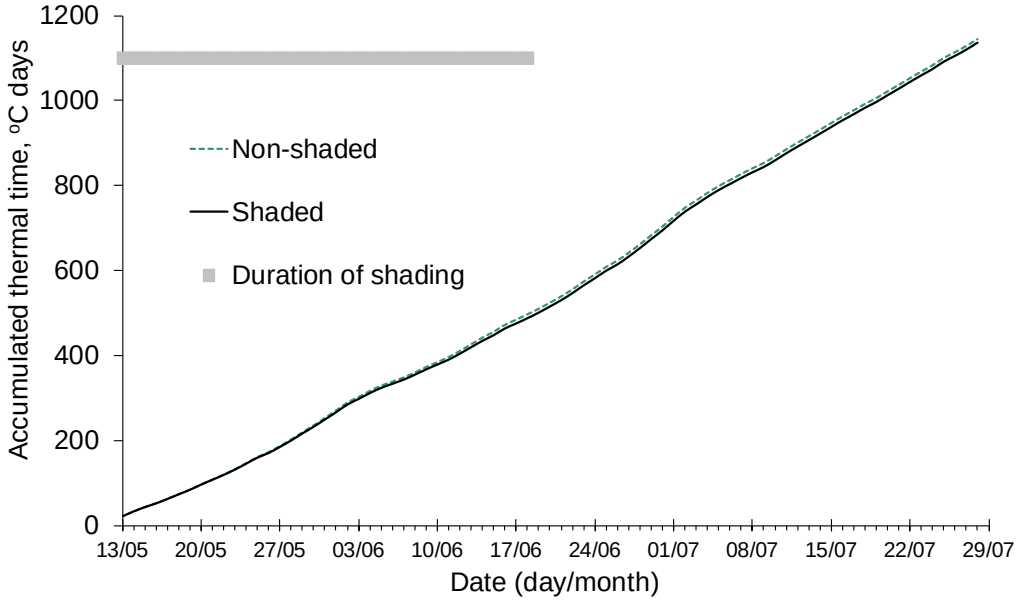

Fig. A-3.2. Accumulated thermal time for shaded and non-shaded plots both during pre-anthesis shading and after shade removal at ADAS 2009. Average daily temperature for shaded and non-shaded plots was 12.8 and 13.1^{o}C respectively during shading and 16.2 and 16.2°C, respectively, after shade removal.

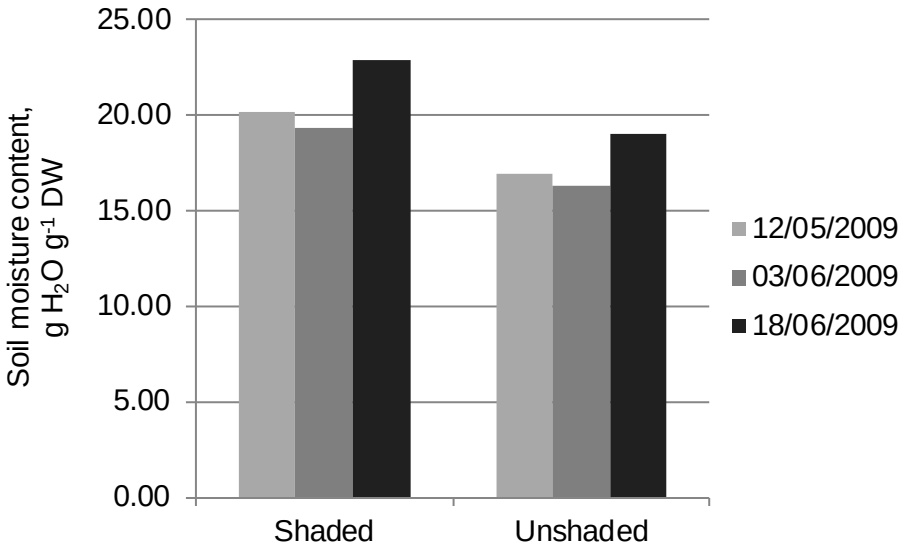

Fig. A-3.3. Gravimetric soil moisture content (mean to 90 cm soil depth) for shaded and non-shaded plots during the period of pre-anthesis shading; ADAS 2009.

Appendix 4.

Table A-4.1. Cross site anova of variety and fungicide effects on the quantity of endogenous ramularia and rhynchosporium DNA (pg DNA 100 μg^{-1} total DNA) in leaf samples at GS59. The top 4 leaf layers were bulked for DNA analysis by qPCR. Data were log10 (x+1) transformed prior to analysis. Values are treatment and interaction means after back-transformation. LSD values are the transformed values.

Factor/Interaction		Ramularia DNA				Rhynchosporium DNA			
Site (S)		SRUC Ab	SRUC Ed	ADAS		SRUC Ab	SRUC Ed	ADAS	
		15.63	5.35	1.25		3.50	0.38	2.38	
Variety (V)		Garner	Quench	Westminster		Garner	Quench	Westminster	
		5.90	5.95	3.97		1.68	2.78	1.07	
Fungicide (F)		Untr	T1	T2	T1 + T2	Untr	T1	T2	T1 + T2
		17.49	5.47	3.56	1.70	8.12	1.37	1.19	0.22
S*V		Garner	Quench	Westminster		Garner	Quench	Westminster	
	SRUC Ab	17.58	20.09	10.72		3.09	3.74	3.70	
	SRUC Ed	5.11	5.90	5.10		0.12	1.19	0.06	
	ADAS	1.89	1.31	0.71		3.21	4.19	0.77	
S*F		Untr	T1	T2	T1 + T2	Untr	T1	T2	T1 + T2
	SRUC Ab	32.19	23.32	15.94	4.57	22.44	3.52	1.40	0.61
	SRUC Ed	34.97	3.69	2.71	1.62	1.77	0.11	0.15	0.01
	ADAS	4.28	1.38	0.51	0.36	10.69	1.64	2.78	0.11
V*F		Untr	T1	T2	T1 + T2	Untr	T1	T2	T1 + T2
	Garner	25.73	5.98	3.85	1.50	6.01	1.78	1.04	0.30
	Quench	20.68	5.95	4.82	1.67	29.62	1.56	1.16	0.20
	Westminst	9.91	4.60	2.37	1.96	2.55	0.86	1.37	0.17
S*V*F		Untr	T1	T2	T1 + T2	Untr	T1	T2	T1 + T2
SRUC Ab	Garner	36.58	39.36	15.60	3.73	23.10	3.09	0.61	0.77
	Quench	41.17	21.03	32.96	5.24	30.77	3.34	1.31	0.59
	Westminst	22.12	15.22	7.63	4.86	15.83	4.20	2.73	0.50
SRUC Ed	Garner	49.12	2.54	2.13	1.51	0.24	0.22	0.01	0.03
	Quench	45.13	3.53	3.86	1.25	13.49	0.08	0.47	0.01
	Westminst	19.23	5.43	2.34	2.18	0.19	0.04	0.01	0.01
ADAS	Garner	9.14	1.38	1.19	0.32	10.56	3.32	4.18	0.21
	Quench	4.26	2.36	0.19	0.36	61.37	2.57	1.99	0.08
	Westminst	1.77	0.69	0.32	0.40	1.22	0.18	2.51	0.05
		P	rep.	l.s.d.	d.f.	P	rep.	l.s.d.	d.f.
S		<0.001	48	0.220	9	0.030	48	0.3724	9
V		0.038	48	0.125	18	0.036	48	0.1942	18
F		<0.001	36	0.136	81	<0.001	36	0.2063	81
S*V		0.416	16	0.267	22	0.210	16	0.4386	20
S*F		<0.001	12	0.286	35	0.045	12	0.4628	30
V*F		0.165	12	0.235	96	0.004	12	0.3592	95
S*V*F		0.425	4	0.434	92	0.755	4	0.6795	84