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Development and field testing of fungicide anti-resistance strategies, with particular reference to strobilurin QoI group of fungicides

by

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ABSTRACT

The development of fungicide resistance in plant pathogen populations has become a major problem since the introduction and widespread use of single-site mode of action fungicides. The overall aim of this project was to provide a scientific basis for improved fungicide resistance management by testing hypotheses relating to dose and frequency in exerting selection pressure for fungicide resistance.

The focus of the project was on the widely used strobilurin (QoI) fungicides that were introduced in 1997 but quickly encountered resistance problems with some pathogens. The initial experimental system used was powdery mildew of barley (*Blumeria graminis* f.sp. *hordei*), in which QoI resistance had already been reported, and subsequently Septoria leaf blotch of wheat (*Septoria tritici*) where resistance emerged during the course of the project. Field trials designed to test the three factors most likely to influence selection for resistance, viz fungicide dose, number of applications, and mixture or alternation with fungicides with a different mode of action, were done at sites in England and Scotland. Samples were taken at different times in the season and tested using a sensitive real-time PCR assay that determines the proportion of the pathogen population carrying the strobilurin-resistant form(G143A) of the mitochondrial cytrochrome *b* target protein.

A countrywide survey was also done to monitor the incidence and distribution of strobilurin resistance in *S. tritici* populations from commercial wheat crops.

Results from the powdery mildew trials showed that both dose and the number of applications influenced resistance development, with higher doses and additional sprays selecting in a stepwise manner for an increased frequency of the cytochrome *b* G143A allele. Alternations and particularly mixtures delayed resistance development.

The mutation responsible for the G143A allele was first detected in *S. tritici* isolates from plots at Rothamsted in 2002, and over the following two seasons (2003-4) the frequency of resistance rose from c. 30% to c. 80%, despite implementation of measures to limit strobilurin use to only two sprays per season, mixed with an alternative fungicide. Results from the field trials confirmed this rapid selection for resistance in *S. tritici* in plots receiving strobilurin treatments, and selection was apparently independent of the dose used or number of applications tested. Alternation or mixture of strobilurins with alternative fungicides was ineffective in delaying this selection. Between 2002 and 2005 the performance of QoI fungicides against Septoria leaf blotch showed a dramatic reduction in efficacy.

By comparison, triazoles remain effective, although there are concerns about their long-term efficacy. An integrated approach to disease management, including use of less susceptible cultivars, is necessary to reduce the risk of fungicide resistance and to safeguard the efficacy of single-site fungicides.

SUMMARY

Introduction

The development of fungicide resistance in plant pathogen populations has been a major problem since the use of single-site mode of action fungicides became widespread. Following the introduction of the benzimidazole group of fungicides in the 1970s widespread resistance occurred rapidly in a number of pathogens. The development of resistance to the EBI (ergosterol biosynthesis inhibitor) group of fungicides in wheat and barley mildew followed in the 1980s. Neither of these resistance problems had been predicted. The belief was that because resistant strains were less fit in laboratory tests than wild-type strains the likelihood of field resistance developing was small. As the changes in sensitivity to some EBI fungicides were relatively small, the loss of activity was not complete and in many cases the decreased performance could be overcome by using higher doses, shortening the intervals between applications or by using more effective products from the same fungicide group.

The strobilurin fungicides (now referred to as QoI fungicides) were introduced in 1997 - a group of highly active compounds which were perfect partners or an alternative for the EBI fungicides (e.g. morpholines and triazoles) which until then had dominated the market. However, the development of resistance to QoI fungicides in wheat mildew (*Blumeria graminis* f. sp. *tritici*) populations occurred very quickly – initially in Germany and then throughout NW-Europe, threatening the future of this group of fungicides. Unlike resistance to the EBI fungicide groups, resistance to the QoI fungicides is regarded as a single step shift resulting in almost complete loss of fungicidal activity. Consequently, when resistance to QoI fungicides occurs in pathogen populations the use of the fungicide group against that disease is often no longer of any value. For this reason, resistance management with the QoIs is particularly important for the industry. However, the QoIs are only the latest group of fungicides to have been commercially developed and further new areas of fungicide chemistry will be developed in the future. Such novel chemistry is also likely to encounter resistance problems unless robust anti-resistance strategies can be put in place. The current status of resistance development to the QoIs in the powdery mildews, and since 2002 in *Septoria tritici* (teleomorph *Mycosphaerella graminicola*), provided a unique opportunity to use these as model systems to better understand both the way in which resistance develops and how it can be affected by fungicide use.

Historically, the Fungicide Resistance Action Committee (FRAC) has given advice on anti-resistance strategies. However, scientific evidence to support the proposed strategies is scarce and consequently there has been disagreement within the industry about best practice and as a result many users have not followed the FRAC guidelines. This project aimed to investigate the theory of how fungicide resistance develops in fungal pathogens, and to provide information on which to base advice for the development of robust fungicide programmes. Such strategies should safeguard the activity of current and future fungicide groups

and prolong the useful life of such fungicides. Because QoI resistance was just emerging in some Scottish populations of barley powdery mildew (*Blumeria graminis* f. sp. *hordei*) in the year 2000, this pathogen was initially chosen as the experimental system for study. Information derived from these experiments might then be used to develop anti-resistance strategies for other fungicide groups and pathogens. However, during the summer of 2002 QoI resistance was also detected in populations of *Septoria tritici* at Rothamsted Research and, subsequently, also in Ireland. Because of the serious implications for the industry (Septoria leaf blotch is the most important foliar disease of winter wheat crops in the UK since the mid-1980s with annual losses of up to £30 million), the project was enlarged to include experimental trials on *Septoria* as well as to start an intensive monitoring programme to establish the extent of this new resistance threat in the UK. It was also felt that comparison of the two contrasting cereal diseases would be of value to assess whether they responded in a similar way to selection by fungicides and treatment regimes aimed to delay resistance development.

For various plant pathogenic fungi, including *Venturia inaequalis*, *Mycosphaerella fijiensis*, *Blumeria graminis*, and *Septoria tritici*, QoI-resistance was linked to a single point mutation in the mitochondrial encoded cytochrome *b* gene. This mutation, resulting in an exchange of glycine to alanine at codon position 143 (G143A) of the cytochrome *b*, was reported to confer high resistance levels to QoI fungicides and can therefore affect disease control. Other resistance mechanisms, including the induction of an alternative respiration pathway to bypass the cytochrome bc_1 complex, fungicide efflux pump activity, metabolisation of the active ingredient (kresoxim-methyl) and other mutations in the target gene (e.g. F129L and G143S), have been reported. However, these mechanisms do not confer high levels of resistance and were not found to cause practical disease control failures. Recently, quantitative real-time PCR (Polymerase Chain Reaction) assays using sequence-specific fluorescent TaqMan probes, Molecular Beacons and Scorpions were applied successfully to detect specific alleles. Studies on the development of QoI resistance in populations of *Blumeria graminis* f. sp. *tritici* showed that these techniques can be applied successfully to validate anti-resistance strategies by directly measuring and comparing the frequency of fungicide resistant alleles in populations before and after application of fungicides.

Previous attempts to monitor resistance development in pathogen populations have been based on determining sensitivity phenotypes in bioassays with the fungicides concerned. This approach measured the proportion of isolates from the pathogen population that were resistant to a particular fungicide. These assays are, however, laborious, time-consuming, expensive and not suited for high-throughput testing. By the time resistance is detectable in a population, the frequency is often too high to expect any modification of fungicide use to affect further resistance development. This project further developed and applied real-time PCR techniques to measure the frequency of specific resistance gene(s) directly in pathogen populations in a high-throughput format. Providing a strong relationship exists between gene frequency and the observed field resistance of the same population, these techniques are reliable and provide the opportunity to test a far

larger spore population than would be possible with conventional biological assays, thereby increasing the probability of detecting the presence of resistance at low frequencies. With the increased sensitivity of this method it was hoped that it would be possible to detect the resistance gene in a pathogen population at a stage when fungicide use could be modified successfully to prevent or reduce further resistance development in practice.

Advances in detection and monitoring techniques are of generic value for assessing resistance risk across a range of pathogens and chemical groups. The pathogen systems chosen for study provided ideal models for testing current hypotheses about risk factors, and thereby informing the conceptual framework for the management of resistance in practice. Ultimately this will safeguard the efficacy of diverse fungicide groups and contribute to sustainable disease control.

Aims/objectives of the project

- To develop and apply allele-specific real-time PCR for rapid high-throughput monitoring of fungicide resistance in pathogen populations.
- To provide a scientific basis for improved fungicide resistance management strategies by testing current hypotheses relating to dose and frequency in exerting selection pressure for fungicide resistance using the QoI fungicides.
- To monitor QoI resistance status in plant pathogens with special emphasis on cereal pathogens.
- To provide novel information on the dynamics of QoI fungicide resistance development in response to different treatment regimes, including contrasting doses, mixtures and alternations.
- To provide more accurate predictions of the possible occurrence and development of resistance.
- To assess effects of different anti-resistance strategies and draw up robust guidelines for fungicide use for farmers, advisers and the agrochemical industry.

Results of field trials

Field trials at two geographic sites comprising barley and wheat plots treated with different doses and combinations of fungicides were sampled over four seasons and the frequency of QoI resistant (A143) alleles in *Blumeria graminis* and *Septoria tritici* populations were measured. During 2001-2005, surveys and retrospective PCR testing of stored leaf samples revealed a rapid displacement of the sensitive *Septoria tritici* population by a resistant population almost exclusively consisting of isolates carrying A143 alleles. QoI resistance development in barley powdery mildew populations was stepwise, increased by higher doses and frequencies of strobilurin sprays and, to a certain extent, delayed by alternation with other groups of fungicides and the use of mixtures of fungicides. The selection for Al43 alleles was more rapid in *Septoria tritici*, often completed after a single spray irrespective of dose. While in this case anti-resistance strategies

based on fungicide mixtures and alternations were not effective in delaying QoI resistance development, mixtures, in particular, ensured improved disease control.

Between 2002 and 2005 the performance of QoI fungicides against Septoria leaf blotch showed a dramatic reduction in efficacy. By comparison, triazoles remained effective, although there are concerns about their long term efficacy. Good farming practices should include use of resistant cultivars and the correct timing of fungicide sprays when needed. The use of fungicides mixtures with each component at recommended/robust dose rate will ensure optimal disease control and reduce the risk of resistance development through multiple modes of action and by keeping the population size small. This strategy should also be used from the outset with any new fungicide with a single mode of action.

Conclusions

- Robust allele-specific real-time PCR assays were developed to quantify fungicide resistance alleles in fungal leaf populations. Provided a clear phenotype-to genotype relationship exists these assays are ideally suited for rapid high-throughput screening of fungicide resistance in field populations.

- Fungicide sensitivity testing linked with cytochrome *b* sequence analysis showed that high levels of QoI resistance in isolates of *Blumeria graminis* and *Septoria tritici* were always associated with the presence of a specific mutation in the mitochondrial cytochrome *b* gene. This mutation, G143A, results in replacement of glycine by alanine at codon 143. Mixtures of G143 (S) and A143 (R) - alleles were never found in single spore isolates during this study.

- For barley powdery mildew, selection for R-alleles increased with both dose and number of sprays with QoI fungicides. Higher rates selected more quickly for R-alleles, but this effect was smaller when the rate of Amistar exceeded 1.0 l/ha. The frequency of R-alleles increased after each spray application. Fungicide mixtures were generally more effective than alternations in slowing resistance development and also improved disease control. At Terrington, the level of green leaf area (GLA) was positively linked with increased spray number, even in the absence of disease. No significant increase in GLA was observed in trials conducted in Scotland, suggesting that climate might play an important role in this physiological effect.

- Retrospective PCR testing of leaf populations of *Septoria tritici* sampled in the absence of and after exposure to QoI fungicides at Rothamsted in 2001 showed that G143A was already present in the UK, albeit at a very low frequency, below 3 %, in untreated populations at the end of the growing season. QoI-resistant isolates, all carrying A143 alleles, were for the first time isolated at Rothamsted during summer 2002.

- Surveys showed that G143A was widely and unevenly spread in populations sampled throughout the UK during spring 2003 with an average R-allele frequency of 28 %. In spring 2004, high R-allele frequencies were detected all over England and Scotland with an average R-allele frequency of 88 %. Asexually-produced conidia spread R-alleles over short distances, within a few metres, while sexual ascospores appear to be more important for the spread of R-alleles over longer distances. There was no obvious cultivar effect on the level of QoI resistance detected in populations.

- By comparison with powdery mildew populations, once resistance was detected selection for R-alleles occurred more quickly in populations of *Septoria tritici* after exposure to QoI fungicides. Generally, fungicide efficacy was so high that a single spray, even at the lowest rate, was sufficient to complete selection for the resistant sub-population. Only at the Dundee site in 2003, was it possible to observe increased R-allele frequencies when higher rates and more sprays of Comet were applied.

- An unexpected positive effect of dose of Comet on disease control, GLA and yield was observed at Terrington in 2004, where approximately 76 % of the Septoria population already carried R-alleles at the start of the season. This could be due to partial control of resistant isolates.

- While the rate of resistance development was hardly affected, fungicide mixtures were generally more effective than alternations and gave improved disease control.

- In comparison to triazoles, the performance of QoI fungicides against Septoria showed a dramatic reduction in efficacy between 2002 and 2005. While triazoles are still very effective, there are now concerns about their future efficacy. Good farming practice should include use of more resistant cultivars and correct timing of fungicide treatments where needed, using mixtures with each component at recommended/robust dose rate to ensure optimal disease control. This will reduce the risk of resistance development through multiple modes of action and by keeping the pathogen population size small. Continuous resistance monitoring is essential to check if each mixture component contributes to disease control and to alleviate the pressure on resistance development towards the other components.

- Resistance to single-site inhibitors is likely to occur, sooner or later. Anti-resistance strategies should therefore be used from the outset with any new fungicide. Once resistance has occurred, it may be too late to delay its rapid development.

TECHNICAL REPORT

1.0 MATERIALS AND METHODS

1.1 Techniques

During this project many different techniques and methods have been developed and/or refined. The most important techniques and methods are summarised here.

1.1.1 Barley powdery mildew isolation and bioassays (BASF protocol)

Upon arrival the sample leaves were cut into suitable lengths and placed in Petri dishes containing 0.4 (w/v) % water agar + 40 ppm benzimidazole + 30 ppm streptomycin to allow fresh conidial sporulation to occur (1 to 4 days). The cut ends of the samples were inserted into the agar. Barley seedlings (cv. Golden Promise) were grown in the greenhouse in plastic pots (20-25 seedlings/pot) for 8-10 days. The seedlings were then treated in a spray cabinet with a solution of Amistar 250 g/l SC in which the concentration of the active ingredient azoxystrobin was adjusted to 50 ppm. Control plants remained untreated. The fungicide treated seedlings were left overnight to dry. Segments (5.5 cm long) were then cut from the seedling leaves and these were then placed into plastic Petri dishes containing 0.4% water agar + 40 ppm benzimidazole + 30 ppm streptomycin. Five leaf segments/Petri dish were laid on the agar with their cut ends inserted into the agar. Five replicate Petri dishes were prepared giving a total of 5 x 5 = 25 leaf segments. The Petri dishes containing leaf segments were then placed with their lids removed into the bottom of an inoculation settling tower. Leaves from the incubated field samples that had produced fresh conidia were then pooled and used as inoculum. They were placed into the top of the inoculation tower and by means of a gentle stream of air the conidia were dislodged into the tower and settled onto the leaf segments contained in the Petri dishes. The lids were then replaced and the Petri dishes were incubated for 7 days at 18°C with 12 h light/darkness (3000 lux). The leaf segments were then assessed by counting the number of powdery mildew pustules that developed on them. The number developing on segments treated with the discriminatory dose of 50 ppm azoxystrobin was compared with the number developing on the untreated segments and the percent resistant conidia in the sample calculated. A variation of the above method was necessary with samples that only produced very little fresh sporulation after incubation for up to 4 days. In these cases conidia from individual pustules were transferred with a small metal hook to sprayed leaf segments and also to control untreated segments. In the results this method is shown as “individual pustule test”.

1.1.2 Isolation of *Septoria tritici* strains and fungicide sensitivity testing

After sampling, leaf segments containing lesions with pycnidia were washed and stapled onto round filter paper. Filters were placed in Petri dishes and wetted without an excess of water. After 24 h incubation at 20^{o}C in the dark, oozing cirri from individual pycnidia were detached with pointed clock maker forceps under a microscope and suspended in 30 µl of sterile water. Using a loop a few microlitres of suspension was

plated out on Yeast Potato Dextrose (YPD) agar amended with 100 µg/ml of both penicillin G and streptomycin and incubated for 5 days in the dark at 20°C. To obtain pure cultures, isolates from single colonies were sub-cultured twice on agar. Spore suspensions of isolates were either used directly in fungicide sensitivity assays after counting spore numbers under a microscope or stored in 50 % (v/v) glycerol at -80°C.

Assays were performed in clear sterile flat-bottom polystyrene microtitre plates (Costar, Corning, NY). Liquid Czapek Dox (Oxoid Ltd.) amended with 5 g/l yeast extract (Oxoid Ltd.) and different concentrations of technical grade of inhibitor/fungicide were used as growth medium. Aliquots of 50 µl of spore suspensions (10^5-10^6 spores/ml) were added to 150 µl growth medium in each well. After three days incubation at 20°C, growth was measured in a MRX plate reader (Dynex Technologies, Chantilly, VA) at 630 nm. After curve fitting of OD readings, EC_{50} values were calculated using dose response relationship curve fitting.

1.1.3 DNA extractions and quantification

DNA was extracted directly from mycelium or leaves by powdering the samples in liquid nitrogen using a pestle and a mortar or, alternatively, crushing the leaves with a Pohlähne roller press whilst adding sufficient DNA extraction buffer. To each powdered sample, DNA extraction buffer consisting of 40 µl 1% (v/v) β-mercapthoethanol, 400 µl TEN buffer (500 mM NaCl, 400 mM Tris-HCl, 50 mM EDTA, 5 mM 1,10-phenanthroline monohydrate, 2 % (w/v) polyvinylpyrrolidone; pH 8.0) and 400 µl 2% (w/v) SDS was added. For larger wheat leaf samples, the amount of DNA extraction buffer to be added was increased until the mixture could be poured. After incubating the mixture for 30 min at 70°C, 400 µl ice-cold ammonium acetate (7.5 M) was mixed with the heat-treated sample and the total suspension kept on ice for 30 min. After centrifugation at 10,000 rpm for 10 min, an equal volume of cold (-20°C) isopropanol was added to the supernatant and the extract shaken at room temperature for 15 min. After centrifugation at 6,000 rpm for 5 min, DNA pellets were washed with ice-cold 70% (v/v) ethanol, centrifuged again and dissolved in 500 µl sterile distilled water.

Thiazole orange is a cyanine dye that specifically binds with dsDNA and can be used for fluorimetric detection and quantification of DNA. From each crude DNA extract and calibration sample with a known amount of DNA, 2 µl of sample was incubated at room temperature in a microtitre plate well with 150 µl of a 2.5 µM thiazole orange solution (1 mM EDTA, 10 mM Tris-HCl, pH 8.0). Fifteen min after excitation at 480 nm, fluorescence was measured at 530 nm using a FLx800 fluorimeter (Bio-Tek Instruments Inc., Winooski, VT) and DNA concentrations calculated using appropriate calibration curves generated from the calibration sample measurements.

1.1.4 Cloning and sequencing of cytochrome *b* genes

Initially, a 675 bp fragment of the cytochrome *b* gene of *Septoria tritici* and *Blumeria graminis* f. sp. *hordei* was amplified with primers CBF1 (5'-TATTATGAGAGATGTAAATAATGG-3') and CBR3 (5'-CCTAATAATTTATTAGGTATAGATCTTA-3'). For *Septoria tritici,* PCR products encompassing the remainder of the *cytb* gene coding region (upstream and downstream of the known sequence) from ST1, a QoI-sensitive strain, were amplified using the Universal GenomeWalker kit (Clontech, Palo Alto, CA). Reactions were carried out according to the manufacturer's instructions. Fragments amplified from GenomeWalker libraries were cloned into pGEM-T easy vector (Promega Corporation, Madison, WI) and sequenced using a dideoxy chain termination method reaction. Primers StcbF1 (5'-CGTAGCAATGGAGGTACGCT-3') and StcbR1 (5'-ATCTTCCGTAAGATGGTATCTCT-3'), annealing to beyond the 3'-end of the putative ATG start codon and 5'-end of the TAG stop codon, respectively, were used to amplify the full length cytochrome *b* gene coding region. Standard PCR was carried out in a Biometra T3 thermocycler (Biotron GmbH, Göttingen, Germany) with Red Hot *Taq* DNA polymerase (ABgene, Epsom, UK) under the following conditions: initial denaturation at 94°C for 3 min, followed by 40 cycles of 94°C for 30 s, 54°C for 1 min, 72°C for 1.5 min with a final extension at 72°C for 8.5 min. The complete cytochrome *b* gene of strain ST1 was cloned, sequenced and analysed using the NCBI BLAST server.

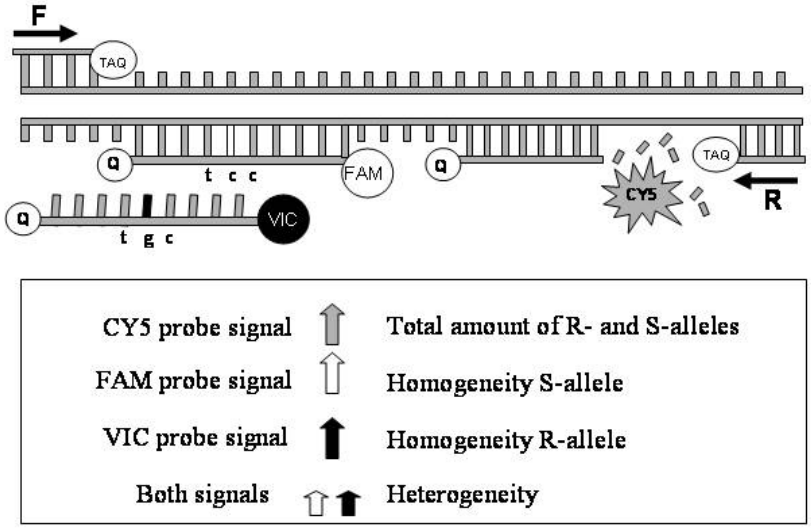
1.1.5 Development of allele-specific real-time PCR assays

Recently, two real-time PCR-based assays have been developed at Rothamsted Research to detect and quantify the G143A mutation conferring strobilurin (QoI) resistance in cereal powdery mildews. One is based on the use of allele-specific primers. During the PCR reaction the amount of amplified product is measured in real-time with the double-stranded DNA intercalating dye SYBR Green I. For this assay two different PCR reactions are needed to detect and quantify QoI resistant (R) and sensitive (S) alleles. The second assay is based on the use of fluorogenic probes and the 5' nuclease assay (TaqMan probe assay). The probes are labelled with both a fluorescent reporter dye and a quencher dye. By using different reporter dyes, cleavage of allele-specific probes can be detected in a single PCR. Based on the different end-point fluorescence values, the proportion of G143 and A143 alleles in a given sample can be estimated. The advantage of the dual-probe-based assays is that both alleles can be detected in one reaction and due to the nature of the reaction (single reaction based on competitive binding of sequence-specific probes) false and/or negative reactions are less likely to occur.

In order to quantify the amount of pathogen DNA and to measure the frequency of QoI-resistant A143 (R-) alleles simultaneously in a single reaction, a PCR assay was developed in which two primers and three probes are acting simultaneously (Figure 1). To obtain specificity, primers Stbf1 (forward) 5'-ACATTAACATGAACAATCGGTACTATAATACTAG-3' and Stbr2 (reverse) 5'-GGATTTCCTGAACCCGCTG-3', amplifying a 299 bp fragment of the *Septoria tritici* cytochrome *b* gene,

and, PMR1 (forward) 5'-TTACTGCATTCCTGGGTTATGTATTG-3' and PMS1 (reverse) 5'-ACAGAGAAACCTCCTCAAAGGAACT-3', amplifying a 136 bp fragment of the *Blumeria graminis* f. sp. *hordei* cytochrome *b* gene, were designed to have mismatches in the last three bases at the 3' primer ends in comparison with other fungal cytochrome *b* sequences.

Figure 1. Simultaneous detection of QoI-sensitive (G143) and -resistant (A143) cytochrome *b* alleles using three different fluorescent DNA probes. The FAM- and VIC-labelled probes are allele-specific; which is determined by a single nucleotide (guanine to cytosine transversion). F, forward primer; R, reverse primer, Q, quencher; Taq, DNA polymerase. Nucleotide sequences cct and cgt are complementary to gga and gca, coding for glycine and alanine, respectively.



The specificity was confirmed by testing of a panel of fungi, including *Alternaria infectora*, *Cladosporium herbarum*, *Fusarium graminearum*, *Oculimacula yallundae*, *Rhizoctonia cerealis*, *Rhynchosporium secalis* and *Stagonospora nodorum*; no amplification products were detected. Probe sequences, based on minor groove binder (MGB) TaqMan probe chemistry, were designed using Primer Express Software (Version 7.1, Applied Biosystems, Foster City, CA). PCR reactions were carried out in 20 µl reaction volumes (capped Thermo-Fast 96 PCR Plates; ABgene), consisting of 5 µl DNA sample, 12.5 µl Platinum® Quantitative PCR SuperMix-UDG (Invitrogen Life Sciences, Carlsbad, CA) and 5 µl sterile distilled water containing primers, probes and ROX reference dye. For detection of *Septoria tritici* cytochrome *b* alleles, the concentrations of the primers and probes were 0.5 µM of forward primer, 0.3 µM of reverse primer, 0.1 µM of 5'-CY5/3'-BHQ2 -labelled probe (5'-AATGCAGCTAACACAAACGGTAAAACGA-3') (Proligo, Paris, France), 0.1 µM of FAM-labelled MGB probe (5'-CTGTTGCTCCTCATAA-3') and 0.2 µM of VIC-labelled MGB probe (5'-TTGCTGCTCATAAAG-3') (Applied Biosystems, Warrington, UK). For *Blumeria graminis* f. sp.

hordei, 0.5 μM of forward primer, 0.3 μM of reverse primer, 0.1 μM of 5'-CY5/3'-BHQ2 -labelled probe (5'-CTTGTCCTATTCATGGTATAGCGCTCATTAGG-3') (Biomers, Germany), 0.1 μM of FAM-labelled MGB probe (5'-CACTGGGGTGCAAC-3') and 0.2 μM of VIC-labelled MGB probe (5'-CACTGGGCTGCAAC-3') (Applied Biosystems, Warrington, UK) were used. Reactions were carried out in a Mx3000P Real Time PCR System (Stratagene Europe, Amsterdam, The Netherlands) for 2 min at 50°C, 2 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 60°C. The increase in fluorescence from probes was recorded at 60°C during every cycle. For each sample, the threshold cycle (Ct, cycle at which the increase of fluorescence exceeded the background) for the CY5-labelled probe was determined. Cleavage of this probe correlates with the amount of pathogen DNA because of its binding to both QoI-sensitive G143 (S-) and -resistant A143 (R-) alleles. Plotting known amounts of DNA against Ct values generated standard curves. The resulting regression equations were used to quantify the amount of pathogen DNA in 'unknown' samples. For each sample, the ratio of VIC and FAM signals, indicators for R- and S-alleles, respectively, was determined five cycles after detection with the CY5-labelled probe. Plotting known R-allele frequencies against signal ratios generated standard curves. Resulting regression equations were used to determine R-allele frequencies in 'unknown' samples. For each test, two series of standards (for pathogen DNA quantification and measurement of R-allele frequencies) were included.

1.2 Barley powdery mildew core field experiments

The proposed experiments were designed to test the three factors most likely to influence the evolution of resistance and to be amenable to manipulation in an anti-resistance strategy

- the dose of fungicide applied
- the number of applications
- alternation/ mixture of fungicides with different modes of action.

A number of alternative fungicide strategies in field situations against barley powdery mildew were evaluated. Effects of each strategy on the frequency of QoI-resistant alleles (G143A) in the population and the observed phenotypic occurrence of resistance in the same population were determined using allele-specific PCR assays and bioassays.

Basis for initial experimental design

- Barley mildew was chosen as the target pathogenas this pathogen had a very high likelihood of developing resistance to QoI fungicides (resistance was only detected at low levels in Scotland in 2000) and it was anticipated that the frequency of resistant isolates was extremely low in the UK at the start of the project in 2001. This presented an ideal model system to test anti-resistance strategies.
- Experiments were carried out on the spring barley variety, Golden Promise - an unusual but necessary choice because it is universally susceptible to all known races of *Blumeria graminis* f. sp. *hordei* and would therefore not impose any pre-selection for genetic variability in the population.

The spring barley was sown into an area surrounded by mildew-susceptible winter barley to ensure that mildew infection occurred early in the life of the spring barley crop. This allowed the maximum number of asexual generations of the mildew population and thus maximised the likelihood of fungicide resistance development.

- Azoxystrobin was used as the test QoI fungicide as it exerts only slight selection pressure, rather than trifloxystrobin or kresoxim-methyl, which would exert very high selection pressure and give very high levels of control, removing most of the pathogen population which we would want to monitor. Experiment treatments were carefully designed to ensure that different doses or number of sprays did not markedly affect the selection pressure (disease control) imposed by the different treatments..

QoI resistance development: effect of dose, application numbers and alternation

All treatments of the field experiment are given in Tables 1 and 2. To study the effect of dose, Amistar (active ingredient azoxystrobin) was used at a range of doses. The influence of application number on the development of resistance was studied by comparing the effects of a single spray with multiple spray programmes (Table 1). Corbel (a.i. fenpropimorph, an EBI inhibitor), was used to study the effect of mixing and alternation on QoI resistance development (Table 2).

Table 1: Effect of dose and application number on QoI resistance development in barley powdery mildew

Treatment number	**Number of sprays**	**Dose per spray[1] (l/ha Amistar)**	**Target level disease control (%)[2]**
1	Nil	Nil	0
2	1	1.0	80
3	1	2.0	95
4	1	3.0	99
5	2	0.5	80
6	2	1.0	95
7	2	1.5	99
8	3	0.3	80
9	3	0.6	95
10	3	1.0	99

[1] Amistar at 1.0 l/ha is equal to 250 g a.i. azoxystrobin/ha

[2] Disease control levels were estimated through extrapolation of data obtained from ADAS fungicide dose-response trials.

Table 2: Effect of mixtures and alternations on QoI resistance development in barley powdery mildew

Treatment number	Sequence and treatment[1]			
11	A	B	A	B
12	B	A	B	A
13	A	A	A	A
14	B	B	B	B
15	A+B	A+B	A+B	A+B
16	Untreated control			

[1]Amistar (A) = 0.5 l/ha, Corbel (B) = 0.25 l/ha; Amistar at 0.5 l/ha is equal to 125 g a.i. azoxystrobin/ha, Corbel at 0.25 l/ha is equal to 175 g a.i. fenpropimorph/ha

Large-scale plots of the susceptible cv. Golden Promise (at least 12 m by 9 m) were used to minimise the inter plot transfer of inoculum. A randomised block design incorporating the 16 treatments with four replicates was used for all experiments. Barley cultivar Riviera was used as surrounding buffer area, with at least 12 m between the trial plots. Once the spring crop had mildew established on it and first sprays had been applied, the surrounding crop was sprayed conventionally with a non-QoI programme to minimise the risk of non-target diseases such as rhynchosporium leaf blotch, rust or net blotch infecting the trial sites.

Experiment site locations

For the 2001/02 growing season, three different sites were used: Terrington-St. Clement, Kings Lynn, Norfolk (ADAS), Boghall Farm (March Park), Biggar Road, Edinburgh (SAC) and Findon Mains, Inverness, Stirling (SAC). Inverness was chosen because QoI resistance was detected here in barley powdery mildew populations sampled in the previous year. For the 2002/03 growing season, field trials were repeated at Terrington St. Clement, Kings Lynn, Norfolk (ADAS) and Boghall Farm (Crofts Field), Biggar Road, Edinburgh.

Sampling and testing

The factors to be measured in the trials are the frequency of resistant alleles, the *in vivo* sensitivity of selected samples and the disease control achieved. The level of disease control is critical in determining the selection pressure exerted by each treatment and was for each sample assessed visually using a sample key according the HGCA recommended list cereal trials protocol. For allele-specific PCR assays, DNA was extracted from samples consisting of 25 leaves infected with fresh powdery mildew pustules using a pestle and mortar. Leaves were sampled from the centre of each plot before and two weeks after each treatment. Leaves from untreated plots were sampled at the same time. For each sample, 50 ng of total DNA was tested in each PCR test. Bioassays/fungicide sensitivity tests were carried out with a selection of leaf samples. DNA from resistant (G143A) isolates was further tested in bioassay in order to confirm that G143A was conferring resistance. Isolates tested negative in PCR were further analysed for validation of the PCR results and to check for the occurrence of other resistant alleles or alternative resistance mechanisms.

1.3 Septoria field experiments at Rothamsted (2001-2003)

Winter wheat cultivars Riband and Savannah were grown in six small plots (10 m x 3 m) at Rothamsted in the 2000/01 and 2001/02 growing season, respectively. One plot remained untreated throughout the season and other plots were treated three times at approximately 21-day intervals (GS 33-37, 39-43 and 51-69) with four different triazole fungicides, Bayfidan (83.3 g a.i. triadimenol/ha), Flamenco (83.3 g a.i. fluquinconazole), Opus (83.3 g a.i. epoxiconazole /ha) and Folicur (166.7 g a.i. tebuconazole/ha), and one QoI fungicide, Twist (166 g a.i. trifloxystrobin /ha). For each plot, ten ears and leaves from different leaf layers were collected three weeks after the last spray (GS 69-75). DNA was extracted using a pestle and

mortar and tested with PCR to determine disease levels and frequencies of QoI-resistant alleles. In 2002, the percentage of leaf area affected by pycnidia bearing lesions was measured as well. Extra flag leaves with pycnidia bearing lesions were collected to isolate *Septoria tritici* strains. The fungicide sensitivity levels of these strains were determined in bioassays.

After the detection of QoI-resistance in the previous year, a field trial in the 2002/03 growing season was designed to investigate the role of ascospores in the further spread of QoI-resistant cytochrome *b* alleles. For this, winter wheat (cultivar Savannah) was planted at Rothamsted, Harpenden, UK on 1 October 2002. During early spring, the trial was divided into two plots of 120 X 125 m, located North East (NE) and South West (SW). In each plot, a square of 30 X 30 m was marked out. Two battery-powered seven-day recording volumetric spore traps (Burkhard Manufacturing Co. Ltd., Rickmansworth, UK), with an air throughput of 14.4 m^3/day, were placed in the centre of the squares; the distance between the two spore traps was approximately 120 m. All plants within three meters distance from the traps were removed in order to avoid capture of rain-splash dispersed conidia *Septoria tritici*. Both plots, with exception of the marked squares, received two sprays of a fungicide mixture consisting of Bravo (500 g a.i. chlorothalonil/ha and Opus (125 g a.i. epoxiconazole/ha) at growth stage (GS) 30 (16 April) and 32 (9 May). The central square located in NE plot was left untreated, whereas the central square in the SW plot received two sprays of Twist (250 g a.i. trifloxystrobin/ha) at GS 32 and 39 (3 June). Meteorological data were collected by the UK Met Office-approved monitoring station at Rothamsted, located at 1.2 km distance from the field, and supplemented with hourly records from the environment change network sensors, also at Rothamsted. Leaves with symptoms were collected on 22 January (GS 20), 2 July (GS 60) and 17 July (GS 64). Spore traps were operated daily from 15 April (GS 30) till 3 September 2003, a month after harvest. Running of spore traps using Melinex polyester film tapes (Burkhard Manufacturing Co. Ltd.) coated with a mixture of paraffin wax and petroleum jelly, DNA extraction from tapes with Ballotini glass beads (Jencons Ltd., Leighton Buzzard, UK) in a FastPrep machine (Savants Instruments, Holbrook, NY) and counting fungal spores on tapes using microscopy was done as described by Calderon *et al*. (2002). In order to compare ascospore counts using microscopy with quantification of *Septoria tritici* DNA by real-time PCR, daily spore tape surfaces were cut in half and tested separately with both techniques. In order to avoid PCR inhibition, DNA samples extracted from each tape sample were diluted, resulting in a final protocol of dissolving DNA in 300 µl of sterile distilled water and testing 1.5 µl of this solution in PCR.

1.4 Septoria QoI resistance survey and industry partner Septoria trials

After the discovery of QoI-resistant strains of *Septoria tritici* in late summer 2002, an intensive survey was conducted during spring 2003. Many Septoria leaf blotch samples (50 randomly sampled leaves with Septoria lesions) were collected by individual farmers, ADAS consultants and Agrochem industry partners at the start and end of the growing season and sent to Rothamsted Research for further testing.

All agrochem industry partners conducted field trials annually to test the effects of different fungicide programmes on disease control and resistance development in populations of *Septoria tritici*. The design of these experiments included some treatments from the core Septoria leaf blotch experiments and the same sampling protocol to aid comparison. They also provided data on a much wider geographic spread of QoI resistance in *Septoria tritici* populations and additional information on other factors that might influence disease levels and QoI resistance development, such as choice and timing of QoI fungicide sprays and effect of different cultivars. The industry partners also carried out resistance monitoring in *Septoria tritici* and other cereal pathogens within the UK and more widely across Europe. Samples were provided for further analysis where novel resistance phenotypes were suspected.

1.5 Septoria core field experiments in 2003 and 2004

The design of the Septoria leaf blotch experiment was based on the same principles as the barley powdery mildew experiment but using winter wheat cv. Consort due to its susceptibility to Septoria leaf blotch and pyraclostrobin as the QoI fungicide due to the need for eradicant activity in the single spray treatments. In the 2002/03 growing season, two different sites were used: Terrington St. Clement, Kings Lynn, Norfolk (ADAS) and Cunmont, Dundee (SAC). In the 2003/04 growing season, field trials were repeated at Terrington, Norfolk and Kimming Hill, Boghall Farm, Biggar Road, Edinburgh (SAC).

QoI resistance development: effect of dose, application number and alternation

All treatments of the field experiment are given in Tables 3 and 4. To study the effect of dose, Comet (a.i. pyraclostrobin) was used at a range of doses. The influence of application number on the development of resistance was studied by comparing the effects of a single spray with multiple spray programmes (Table 3). Opus (a.i. epoxiconazole), was used to study the effect of mixing and alternation (Table 4).

Table 3. Effect of dose and application number on QoI resistance development in Septoria tritici leaf blotch.

Treatment number	Number of sprays	Dose per spray[1] (l/ha Comet)	Target level of disease control (%)[2]
1	Nil	Nil	0
2	1	1.0	75
3	1	2.0	80
4	1	3.0	90
5	2	0.5	80
6	2	1.0	85
7	2	1.5	90
8	3	0.3	80
9	3	0.6	85
10	3	1.0	90

[1] Comet at 1.0 l/ha is equivalent to 250 g a.i. pyraclostrobin/ha.
[2] Levels of disease control were estimated through extrapolation of data obtained from ADAS fungicide dose-response trials.

Plots were approximately 2 metres wide, 20 metres long and separated from the next plot by a fungicide treated barrier plot. A randomised block design incorporating the 17 treatments with four replicates was used for all experiments. Blocks were separated by at least a 2 metre gap which also received the 'barrier plot' fungicide programme (GS32: Opus 0.5 l (62.5 g a.i. epoxiconazole/ha) + Bravo 1.0 l (500 g a.i. chlorothalonil/ha) + Unix 1.0 kg (750 g a.i. cyprodinil/ha), the latter only if eyespot risk was evident, GS39: Opus 1.0 l + Bravo 1.0 l and GS59: Folicur 0.5 l (125 g a.i. tebuconazole/ha)) in order to prevent splash-borne spores from treated plots contaminating adjacent treated plots.

Table 4. Effect of mixtures and alternations on QoI resistance development Septoria tritici leaf blotch

Treatment number	**Sequence and treatment[1]**			
	GS32	GS33	GS39	GS59
11	Nil	Nil	Nil	Nil
12	B	A	B	A
13	A	B	A	B
14	A	A	A	A
15	B	B	B	B
16	A+B	A+B	A+B	A+B
17	2A+2B	Nil	2A+2B	Nil

[1]Doses of products applied: Comet (A) = 0.25 l/ha, Opus (B) = 0.25 l/ha; Comet at 1.0 l/ha is equal to 250 g a.i. pyraclostrobin/ha, Opus at 1.0 l/ha is equal to 125 g a.i. epoxiconazole/ha

Spray timings and sampling

The spray and sampling timings of the Septoria leaf blotch core field experiments are summarised in Table 5. At GS32, prior to any treatment, 25 plants per plot were collected. At GS39, GS59 and GS75, 25 main tillers per plot were collected at random.

Table 5. Spray and sampling timings of the Septoria leaf blotch core field experiments

	GS32	GS33	GS39	GS59	GS75
1-spray	-	-	✓	-	-
2-spray	✓	-	✓	-	-
3-spray	✓	-	✓	✓	-
4-spray	✓	✓	✓	✓	-
Sampling	Whole plants	none	L2, L3, L4	L1, L2, L3	L1, L2, L3

Assessments

The factors to be measured in the trials were the frequency of resistant alleles, the *in vivo* sensitivity of selected samples and the level of disease present on each leaf layer at each sampling date. The level of disease control is critical in determining the selection pressure exerted by each treatment. A full disease and

GLA assessment (on 10 plants) was carried out at all sampling timings. At GS 39, 59 and 75, leaves from all 25 tillers were collected, separated by leaf layer, and sent in plastic bags to Rothamsted Research for PCR analysis and isolation/fungicide sensitivity testing. For allele-specific PCR assays, DNA was extracted from each sample of 25 leaves. For each sample, 50 ng of total DNA was tested by PCR. For a selection of samples, strains of *S. tritici* were isolated and their QoI sensitivity determined. DNA from resistant isolates was further tested by PCR for presence of QoI-resistant cytochrome *b* alleles (A143) to validate the results and to check if other resistance mechanisms were operating.

1.6 Septoria leaf blotch core field experiments in 2005

The aim of the experiments conducted during the 2004/05 growing season was to determine the level of disease control by QoIs at 'resistant' sites, relative to triazole fungicides and chlorothalonil, and to determine whether disease control was differentially compromised in eradicant versus protectant treatments. A summary of treatments is given in Table 6. Trials with cv. Consort were conducted at Terrington St. Clement, Kings Lynn, Norfolk (ADAS) and Whitesome Hill, Whitsome, Berwickshire, Scotland.

Table 6. Treatments used for the Septoria leaf blotch core field experiment in 2005

	Sequence and treatment[1]			
	GS30	**GS32**	**GS39**	**GS59**
1	-	-	-	Folicur (0.5)
2	Comet (0.5)	Comet (0.5)	Comet (0.5)	Folicur (0.5)
3	Opus (0.5)	Opus (0.5)	Opus (0.75)	Folicur (0.5)
4	Opus (0.5)	Opus (0.5)	Comet (0.5) + Opus (0.75)	Folicur (0.5)
5	Opus (0.5)	Comet (0.5) + Opus (0.5)	Opus (0.75)	Folicur (0.5)
6	Comet (0.5) + Opus (0.5)	Opus (0.5)	Opus (0.75)	Folicur (0.5)
7	Comet (0.5) + Opus (0.5)	Comet (0.5) + Opus (0.5)	Opus (0.75)	Folicur (0.5)
8	Comet (0.5) + Opus (0.5)	Comet (0.5) + Opus (0.5)	Comet (0.5) + Opus (0.75)	Folicur (0.5)
9	-	Comet (0.5) + Opus (0.5)	Comet (0.5) + Opus (0.75)	Folicur (0.5)
10	-	-	Comet (0.5) + Opus (0.75)	Folicur (0.5)
11	-	Comet (0.5) + Opus (0.5)	-	Folicur (0.5)
12	-	-	Opus (0.75)	Folicur (0.5)
13	-	Opus (0.5)	Opus (0.75)	Folicur (0.5)
14	Comet (0.5) + Opus (0.5) + Bravo (1.0)	Comet (0.5) + Opus (0.5)	Comet (0.5) + Opus (0.75)	Folicur (0.5)
15	Comet (0.5) + Opus (0.5) + Bravo (1.0)	Comet (0.5) + Opus (0.5) + Bravo (1.0)	Comet (0.5) + Opus (0.75)	Folicur (0.5)
16	Comet (0.5) + Opus (0.5) + Bravo (1.0)	Comet (0.5) + Opus (0.5) + Bravo (1.0)	Comet (0.5) + Opus (0.75) + Bravo (1.0)	Folicur (0.5)
17	-	Opus (0.5)	Opus (0.75)	Folicur (0.5) + Comet (0.5)
18	-	Opus (0.5)	Comet (0.5) + Opus (0.75)	Folicur (0.5) + Comet (0.5)
19	-	Opus (0.5) + Bravo (1.0)	Opus (0.5) + Bravo (1.0)	Folicur (0.5) + Comet (0.5)
20	-	Opus (0.5) + Bravo (1.0)	Comet (0.5) + Opus (0.5) + Bravo (1.0)	Folicur (0.5) + Comet (0.5)

[1]Rate in l/ha is given for each product; active ingredients used are pyraclostrobin (as Comet/Vivid/Tocana, 250g.ai/l), epoxiconazole (as Opus 125g.ai/l) and chlorothalonil (as Bravo 720g a.i./l)

A randomised block design incorporating the 20 treatments with four replicates was used for this experiment. Plot size was 12 X 2 m.

Sampling and assessments

Foliar disease assessments were carried out on whole plants (GS 30 and GS 32) and on each leaf layer with >50 % green leaf area from 10 tillers at GS 39, 59 and 75. Ten leaves with symptoms from whole plants at GS 30 and 10 leaves from each of the upper three leaves at GS 75 were tested by PCR to determine the level of disease and R-allele frequency.

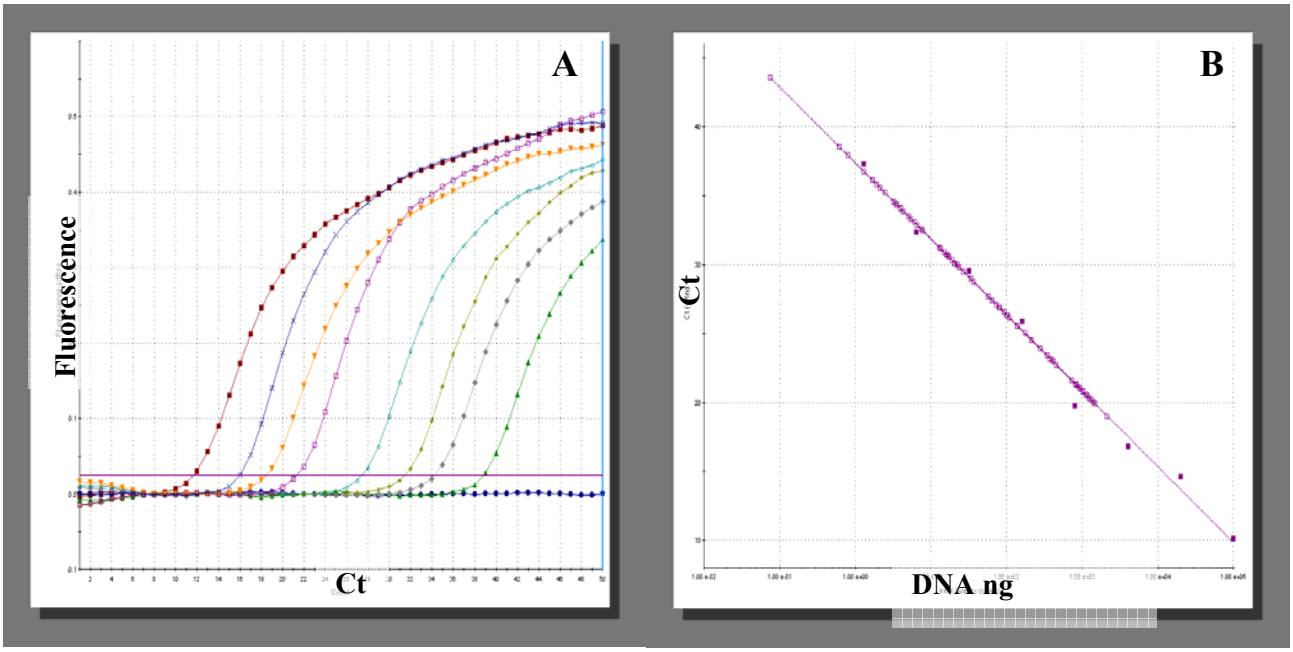
2.0 RESULTS

All raw data collected from the core experiments on barley powdery mildew and Septoria leaf blotch are compiled in Appendix A.

2.1 Application of PCR assays to quantify QoI-resistant alleles in fungal populations

For both *Blumeria graminis* (wheat and barley powdery mildew) and *Septoria tritici* a triple taqMan probe assay was developed. Cleavage of the CY5-labelled probe (Figure 2A) correlates with the amount of pathogen DNA because it binds to both QoI-sensitive G143 (S-) and -resistant A143 (R-) alleles. Plotting known amounts of DNA against Ct values generated standard curves; an example for *S. tritici* is shown in Figure 2B). The resulting regression equations were used to quantify the amount of pathogen DNA in 'unknown' samples (Figure 2B).

Figure 2. Fluorescent PCR-amplication signals for various amounts of *S. tritici* DNA obtained during cleavage of the CY5-labelled probe (A) and the corresponding relationship between pathogen amount and PCR detection cycle (Ct) (B). From left to right decreasing amounts of pathogen DNA (5-fold d ilutions from 100,000 to 1.28 pg), no signal recorded for wheat DNA control (A).



For each sample, the ratio of VIC and FAM signals, indicators for R- and S-alleles, respectively, was determined five cycles after detection with the CY5-labelled probe. The PCR amplification curves for the *S. tritici* R-allele frequency calibration samples are shown in Figure 3. Plotting known R-allele frequencies against signal ratios generated standard curves (Figure 4). Resulting regression equations were used to determine R-allele frequencies in 'unknown' samples.

Figure 3. Fluorescent PCR-amplification signals for calibration samples. Signals obtained during cleavage of CY5 labelled probe (detection of R- and S-alleles) (A), VIC-labelled probe (detection of R-alleles) (B) and FAM-labelled probe (detection of S-alleles) (C). Samples have the same total amount of alleles but contain different ratios of R- and S-alleles (0, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95 and 100 % of R-alleles).

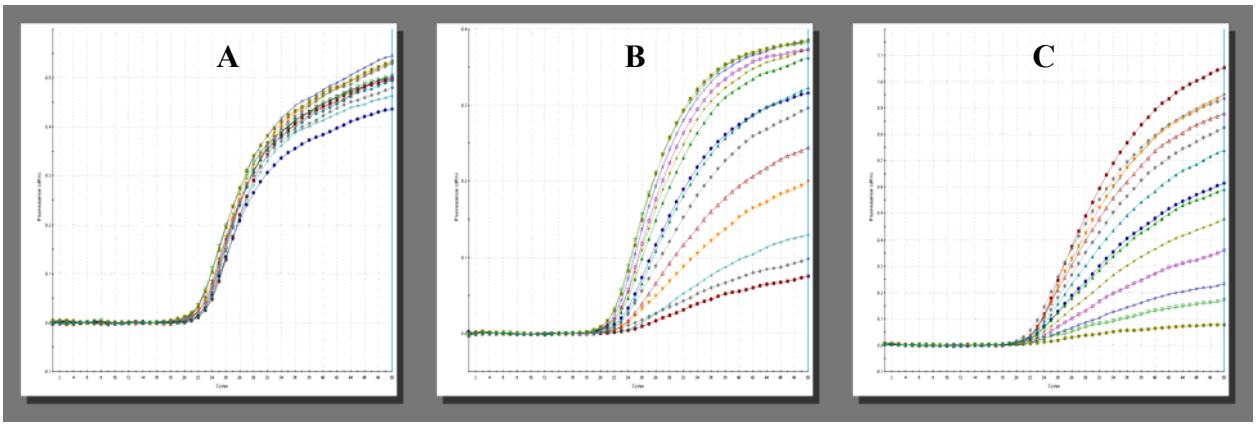
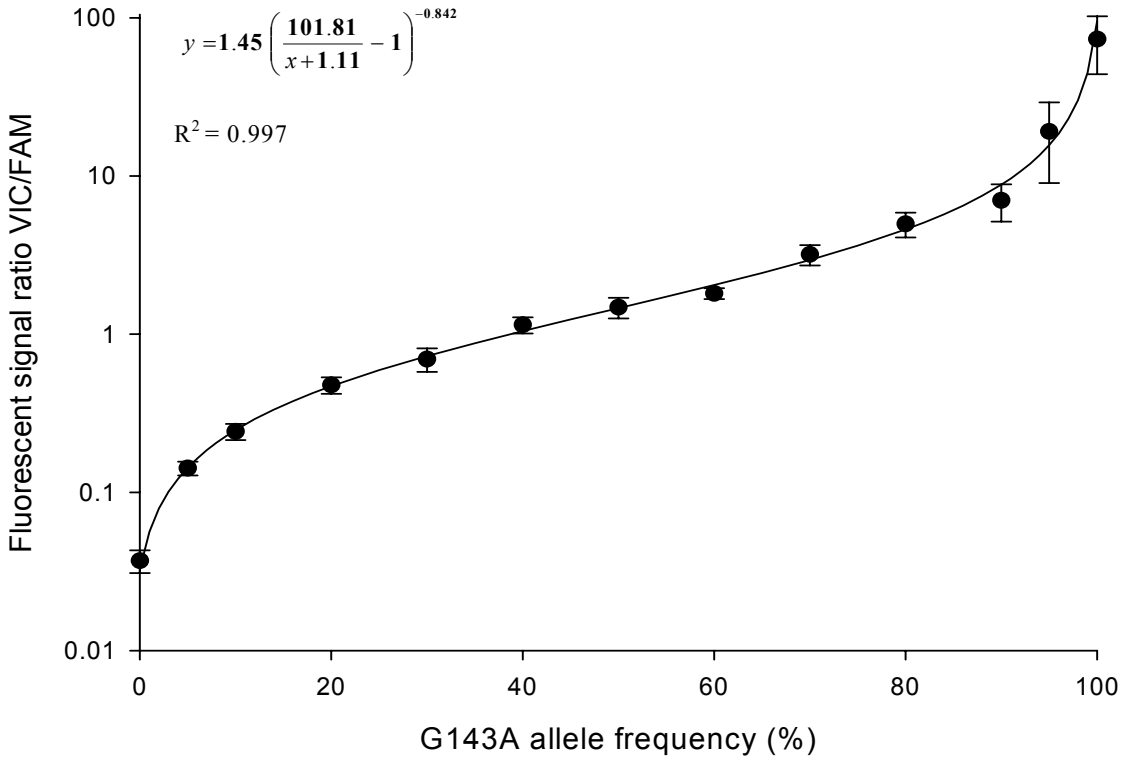


Figure 4. Relationship between G143A frequency and the ratio between the fluorescent signals VIC and FAM, targeting A143 (R-) and G143 (S-) alleles, respectively. Calibration curve presented with standard error bars was derived from four separate experiments with *S. tritici* DNA samples.



Previous research conducted on wheat powdery mildew (*B. graminis* f. sp. *tritici*) (DEFRA Commission PT0226) showed the potential of quantitative real-time PCR diagnostics to study the evolution of fungicide resistance at the population level by directly monitoring the genotype. Because barley powdery mildew is

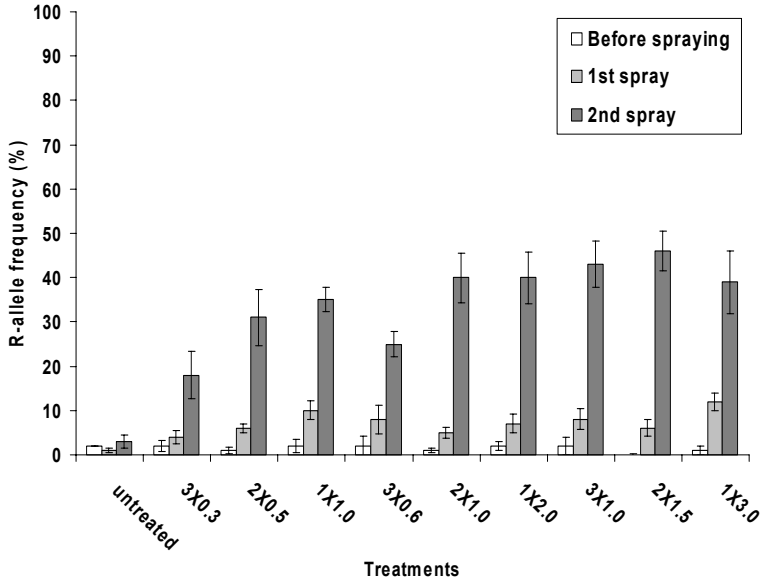
closely related to wheat powdery mildew (sequence of the cloned cytochrome *b* gene fragment is identical), the new assay based on the three TaqMan probes was initially tested on 150 UK wheat powdery mildew isolates obtained in the previous project and a limited set of barley powdery mildew isolates. The results of the TaqMan probe assays were similar to those obtained with the SYBR Green I assays and showed a clear phenotype to genotype relationship. All QoI-sensitive powdery mildew isolates tested were homoplasmic for G143 alleles whereas only A143 alleles were detected in QoI-resistant isolates. A clear relationship was also established for *S. tritici*. Fungicide sensitivity tests and PCR showed that all azoxystrobin-resistant isolates tested, with ED_{50} > 1.0 µg/ml, carried A143 cytochrome *b* alleles. Mixtures of G143 and A143 alleles were not detected in *S. tritici* isolates.

2.2 Barley powdery mildew core field experiments in 2002 and 2003

2.2.1 Effect of dose rate and application number on QoI-resistance developmentLocation Edinburgh (March Park) in 2002

Unfortunately, only two sprays were applied at Edinburgh (March Park) in the first season due to late establishment of powdery mildew infections in the trial plots. However, a clear trend for selection for QoI-resistance was observed in the dose rate experiment (Figure 5).

Figure 5. Effect of fungicide applications on the frequency of QoI-resistant alleles in barley powdery mildew populations at Edinburgh in 2002. R-allele frequencies before and after 1 and 2 sprays of Amistar at different doses (l/ha) are given. Sprays were applied at GS 51 and 60. Standard error bars are shown.



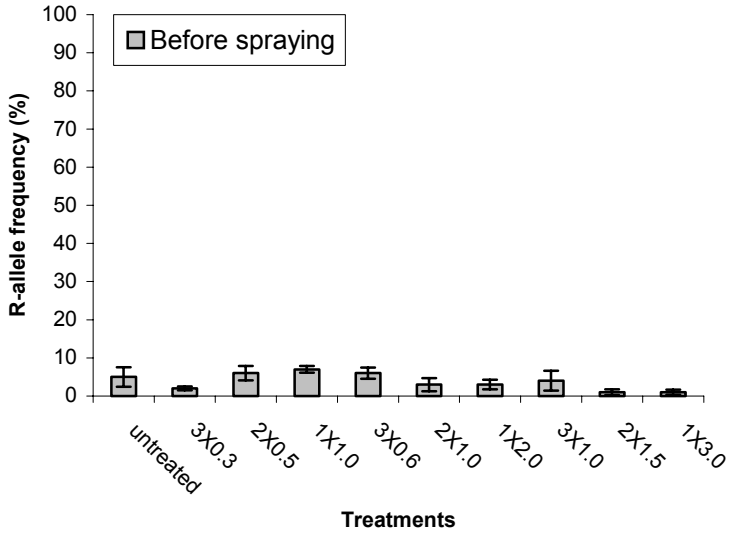
Low R-allele frequencies between 2 and 9 % were measured for powdery mildew populations sampled in 10 out of the 40 plots tested before spraying at GS 51. The average R-allele frequency was approximately 2 %. The R-allele frequency increased only after treatment with Amistar. The selection for R-alleles was gradual

as the effect of a single treatment was more prominent in the populations sampled at the third sample timing (two weeks after the second spray timing and four weeks after the first spray timing). The selection for R-alleles was dose dependent; higher doses selected R-alleles more quickly although the differences between doses were small when the dose exceeded 1.0 l/ha.

Location Inverness (Findon Mains) in 2002

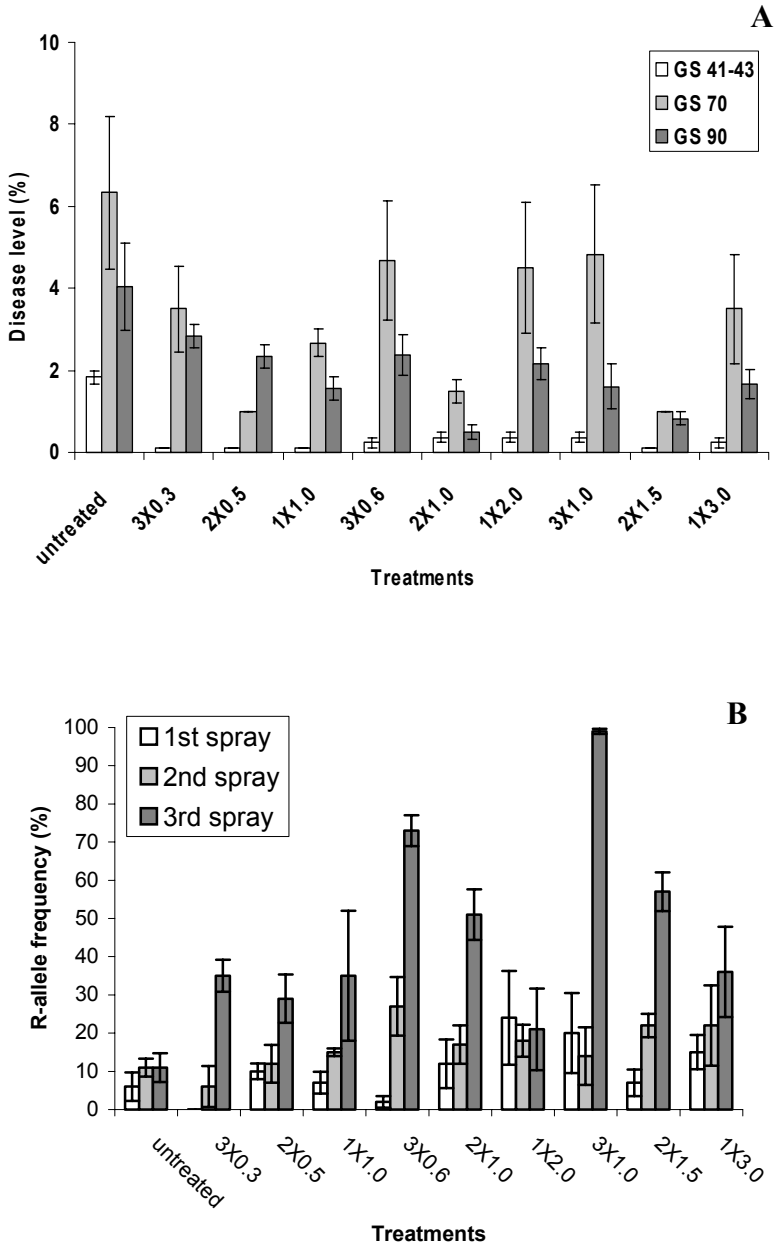
For mildew populations at Inverness, initial R-allele frequencies between 2 and 11 % were measured in 25 out of the 40 plots sampled (Figure 6). The average R-allele frequency was approximately 4 %.

Figure 6. R-allele frequencies measured in mildew populations sampled from different plots at Inverness at GS 26-31 in 2002. Samples were taken before spraying. Standard error bars are shown.



Visual assessments showed that the levels of powdery mildew were much lower in the treated plots than the untreated plot after the first spray at GS 41-43 (Figure 7A) whereas after the second and third spray the differences in disease levels between the untreated and treated plot were much smaller. No significant effect of dose and spray number on the level of disease control was observed. As seen in the mildew population sampled at Edinburgh, R-allele frequencies increased only after treatment with Amistar. Highest R-allele frequencies were measured in populations sampled two weeks after the third spray (Figure 7B).

Figure 7. Effect of fungicide applications on disease levels and R-allele frequencies in barley powdery mildew populations at Inverness in 2002. Disease levels (percentage of leaf area covered with mildew pustules) on Leaf 2 at GS 41-43 (after first spray), Leaf 2 at GS 70 (after the second spray) and Flag at GS 90 (after third spray) (A) and R-allele frequencies after 1, 2 and 3 sprays of Amistar at different doses (l/ha) are given (B). Standard error bars are shown.

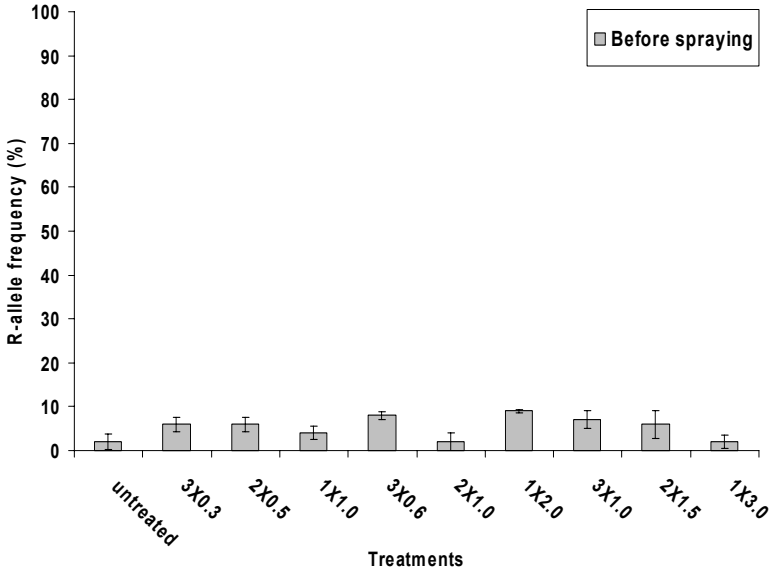


Selection of R-alleles showed a dose dependence; higher doses selected R-alleles more quickly although the differences between doses were smaller when the dose exceeded 1.0 l/ha. The largest differences were observed between 3 sprays of 0.3, 0.6 and 1.0 l/ha of Amistar with average R-allele frequencies of 35, 73 and 99 % respectively. Higher spray numbers also selected R-alleles more quickly as shown for one, two and triple applications of 1.0 l/ha of Amistar where R-allele frequencies of 35, 51 and 99 % respectively were measured.

Location Terrington in 2002

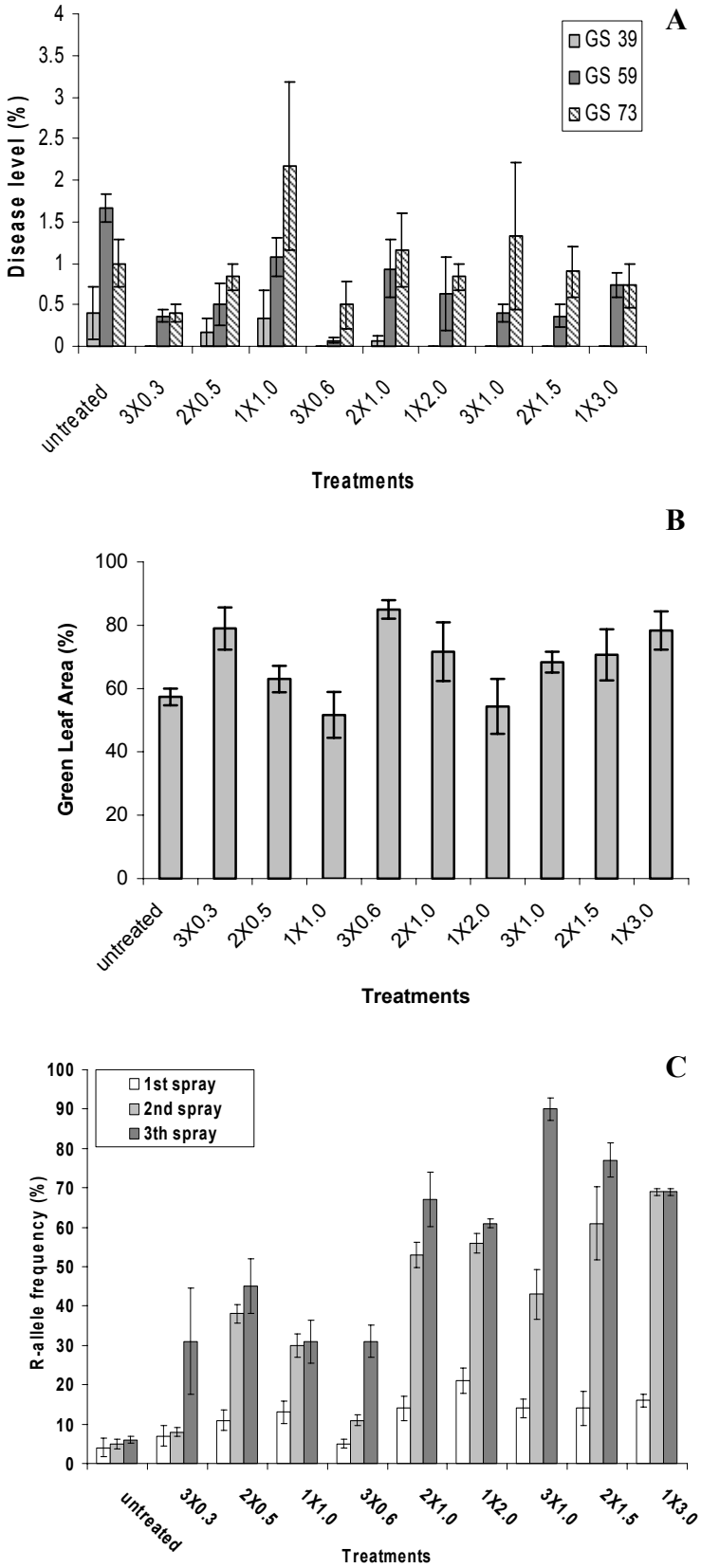
R-allele frequencies between 2 and 11 % were measured in 23 out of the 30 plots sampled before treatment at location Terrington in 2002 (Figure 8). The average R-allele frequency was approximately 5 %.

Figure 8. R-allele frequencies measured for mildew populations sampled from different plots at Terrington at GS 32 in 2002. Samples were taken before spraying. Standard error bars are shown.



Visual assessments showed that the levels of powdery mildew were much lower in the treated plots than the untreated plot after the first spray at GS 39 and the second spray at GS 59 (Figure 9A). After the second spray, high disease levels were also recorded in most plots which had received only a single spray. At GS 73, differences between disease levels amongst plots were less clear although lowest levels of disease were recorded for three sprays of 0.3 and 0.66 l/ha of Amistar. The three times 1.0 l/ha treated plot showed a higher average level of disease than the untreated plot. Green Leaf Area (GLA) assessments (Figure 9B) did not correlate with mildew disease levels, and no other foliar diseases were observed. Spray number appears important for the amount of GLA. Highest levels of GLA were measured with three sprays of Amistar at 0.3 and 0.6 l/ha. As observed for the other locations, R-allele frequencies increased only after treatment with Amistar (Figure 9C). Although R-allele frequencies were still relatively low in comparison to those recorded after the second and third spray, a clear selection for R-alleles was already evident after the first spray. Selection for R-alleles was again dose dependent, with higher doses selecting more quickly for R-alleles. Similar to the other locations, differences between the doses were smaller once the Amistar dose exceeded 1.0 l/ha. Average R-allele frequencies measured for three sprays of 0.3, 0.6 and 1.0 l/ha of Amistar were 31, 31 and 90 % respectively. Higher spray numbers also selected for R-alleles as shown for the single, double and triple applications of 1.0 l/ha of Amistar where R-allele frequencies of 31, 67 and 90 % respectively were measured.

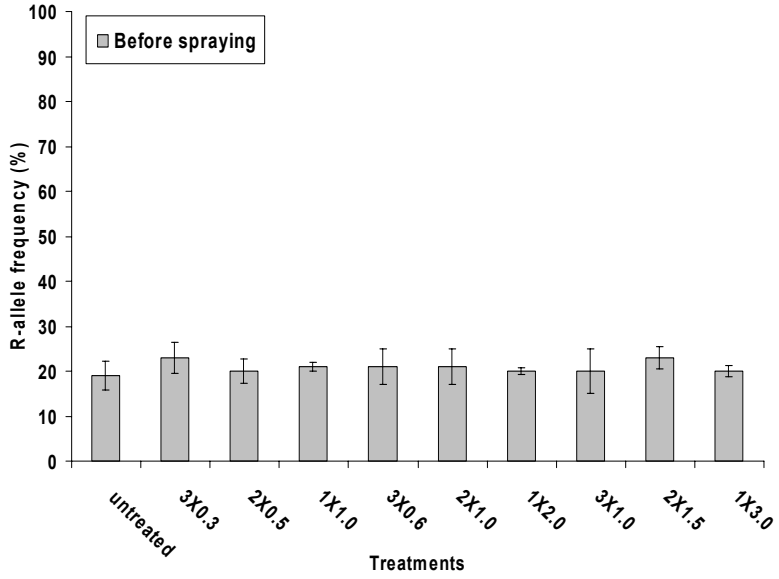
Figure 9 Effect of fungicide applications on disease level, Green Leaf Area (GLA) and R-allele frequency in powdery mildew populations at Terrington in 2002. Disease levels (percentage of leaf area covered with mildew pustules) on Leaf 3 after the 1st (GS 39), 2nd (GS 59) and 3rd spray (GS 73) (A), GLA of Leaf 3 (GS 73) (B) and R-allele frequencies after 1, 2 and 3 sprays of Amistar at different doses (l/ha) are given (C). Standard error bars are shown.



Location Terrington in 2003

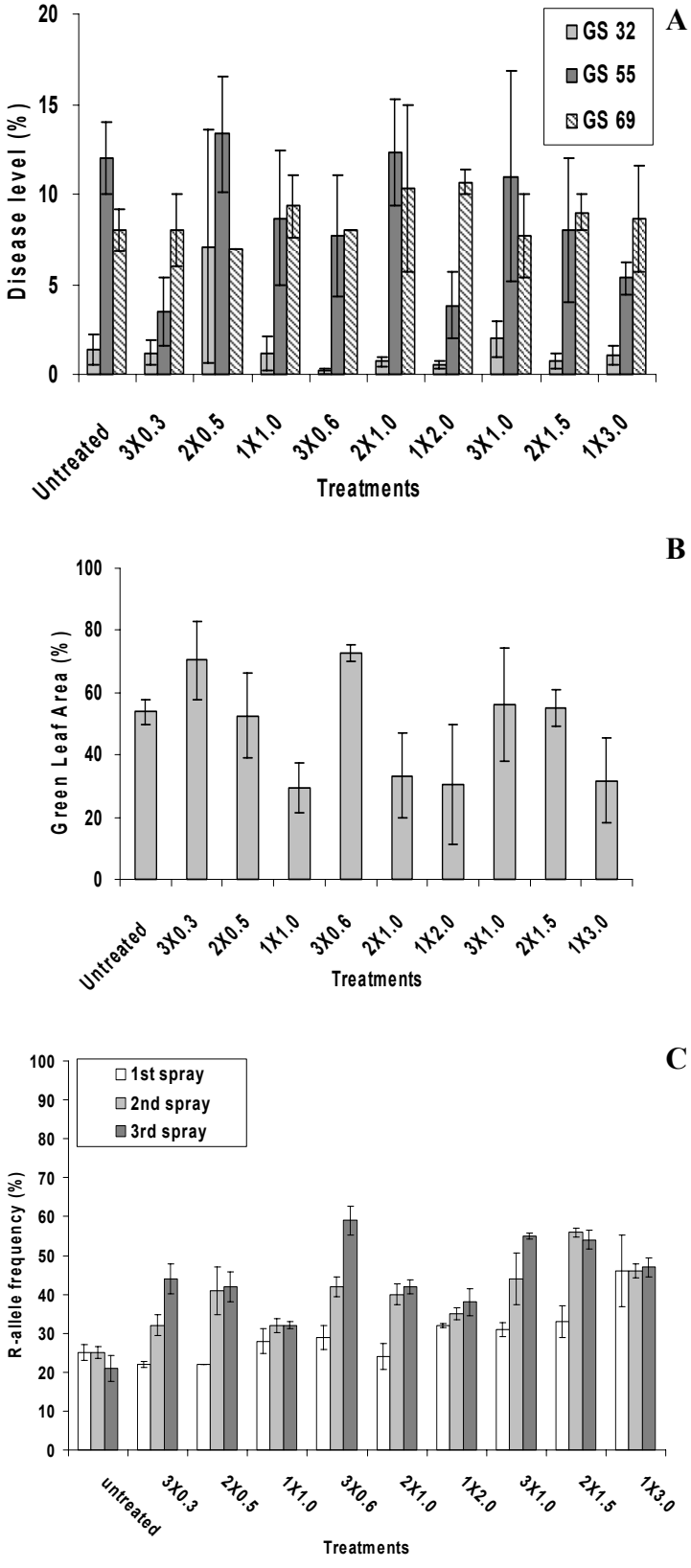
Before spraying, R-allele frequencies between 13 and 30 %, with an average of approximately 20 %, were measured in the 30 plots sampled at Terrington in 2003 (Figure 10).

Figure 10. R-allele frequencies measured for mildew populations sampled from different plots at Terrington at GS 30 in 2003. Samples were taken before spraying. Standard error bars are shown.



Visual assessments showed that levels of powdery mildew were lower in most of the treated plots than the untreated plot after the first spray at GS 32 (Figure 11A). After the second and third spray, no clear trends between spray programme and disease levels were observed; several treated plots had more disease than the untreated plot. Final powdery mildew levels between 1 and 15 % on Leaf 3 were high in comparison to the previous year where disease levels between 0 and 4 % were measured. GLA assessments (Figure 11B) did not correlate with disease levels, and no other foliar diseases were observed. Similar to 2002, spray number seemed important for the amount of GLA. Highest average levels of GLA were measured after three sprays of Amistar (0.3, 0.66 and 1.0 l/ha). R-allele frequencies increased only after treatment with Amistar (Figure 11C). In comparison to results of other experiments, selection of R-alleles was not pronounced with a maximum average R-allele frequency of 59 % in populations sampled after three sprays of 0.66 l/ha of Amistar. Selection of R-alleles was dosedependent; in general higher doses selected R-alleles more quickly. As shown for the other locations, differences between the doseswere smaller when the Amistar dose exceeded 1.0 l/ha. Average R-allele frequencies measured for three sprays of 0.3, 0.6 and 1.0 l/ha of Amistar were 44, 59 and 55 % respectively. Higher spray numbers also selected for R-alleles as shown for the single, double and triple applications of 1.0 l/ha of Amistar where R-allele frequencies of 32, 42 and 55 % respectively were measured.

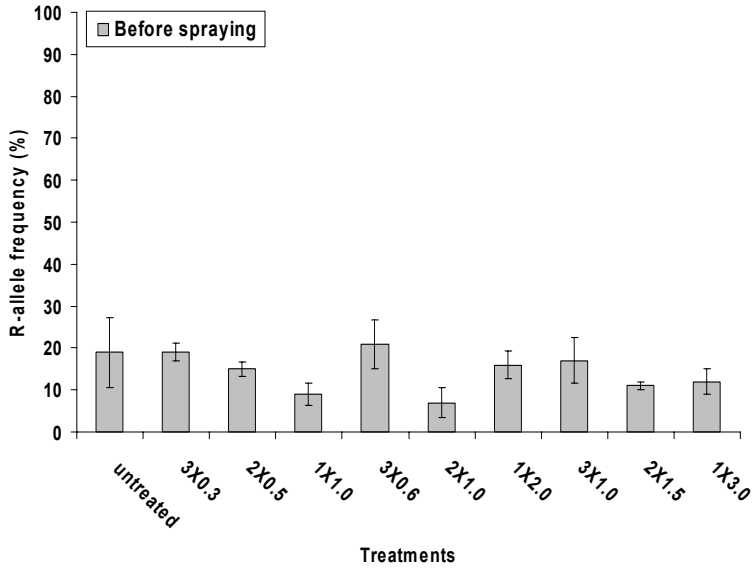
Figure 11. Effect of fungicide applications on disease level, Green Leaf Area and R-allele frequencies in powdery mildew populations at Terrington in 2003. Disease levels (percentage of leaf area covered with mildew pustules) on Leaf 3 after 1st (GS 32), 2nd (GS 55) and 3rd spray (GS 69) (A), GLA of Leaf 3 (GS 69) (B) and R-allele frequencies after 1, 2 and 3 sprays of Amistar at different doses (l/ha) are given (C). Standard error bars are shown.



Location Edinburgh (Crofts Field) in 2003

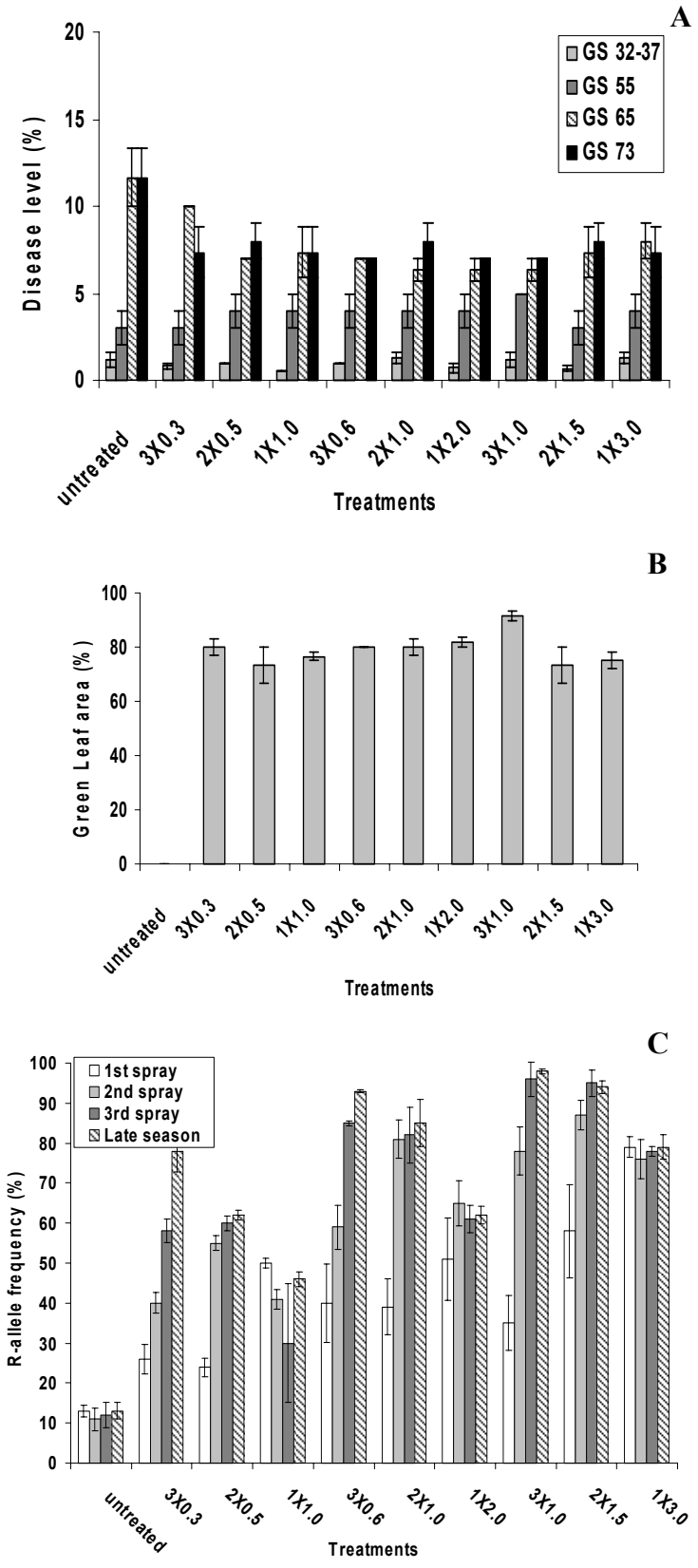
Before spraying, R-allele frequencies between 4 and 35 % were measured in 29 out of the 30 plots sampled at Edinburgh in 2003 (Figure 12). The average R-allele frequency was approximately 15 %.

Figure 12. R-allele frequencies measured in mildew populations sampled from different plots at Edinburgh in 2003. Samples were taken before spraying at GS 31-32. Standard error bars are shown.



Visual leaf disease assessments showed no clear trend between spray programme and disease level; disease levels at GS 32-37 and 55, in untreated plots or after one or two sprays, were very similar (Figure 13A). GLA measurements for Leaf 3 at GS 80 showed no clear differences between treatments (Figure 13B). The R-allele frequencies increased only after treatment with Amistar (Figure 13C). A clear selection for R-alleles was already evident after the first spray. Selection of R-alleles was dose dependent. Average R-allele frequencies in populations exposed to three sprays of 0.33, 0.66 and 1.0 l/ha of Amistar were 58, 85 and 96 % respectively at GS 65. Interestingly, the late season samples, taken four weeks after the third spray at GS 73, showed that fungicides applied were still active as higher proportions of R-alleles were detected with R-allele frequencies of 78, 93 and 98 % in the 0.33, 0.66 and 1.0 l/ha of Amistar treated plots, respectively. Higher spray numbers also selected for increasing R-allele frequencies as shown for the one, two and three applications of 1.0 l/ha of Amistar where R-allele frequencies of 30, 61 and 78 % respectively were measured at GS 65.

Figure 13. Effect of fungicide applications on disease levels, Green Leaf Area and R-allele frequencies in powdery mildew populations sampled from Leaf 3 at Edinburgh in 2003. Disease levels for leaf 3 after the 1st (GS 32-37), 2nd (GS 55) and 3rd spray (GS 65 and 73) (A), GLA at GS 80 (B) and R-allele frequencies after 1, 2 and 3 sprays of Amistar at different doses(l/ha) are given (C). Late season sample was taken 4 weeks after the third spray at GS 73. Standard error bars are shown.

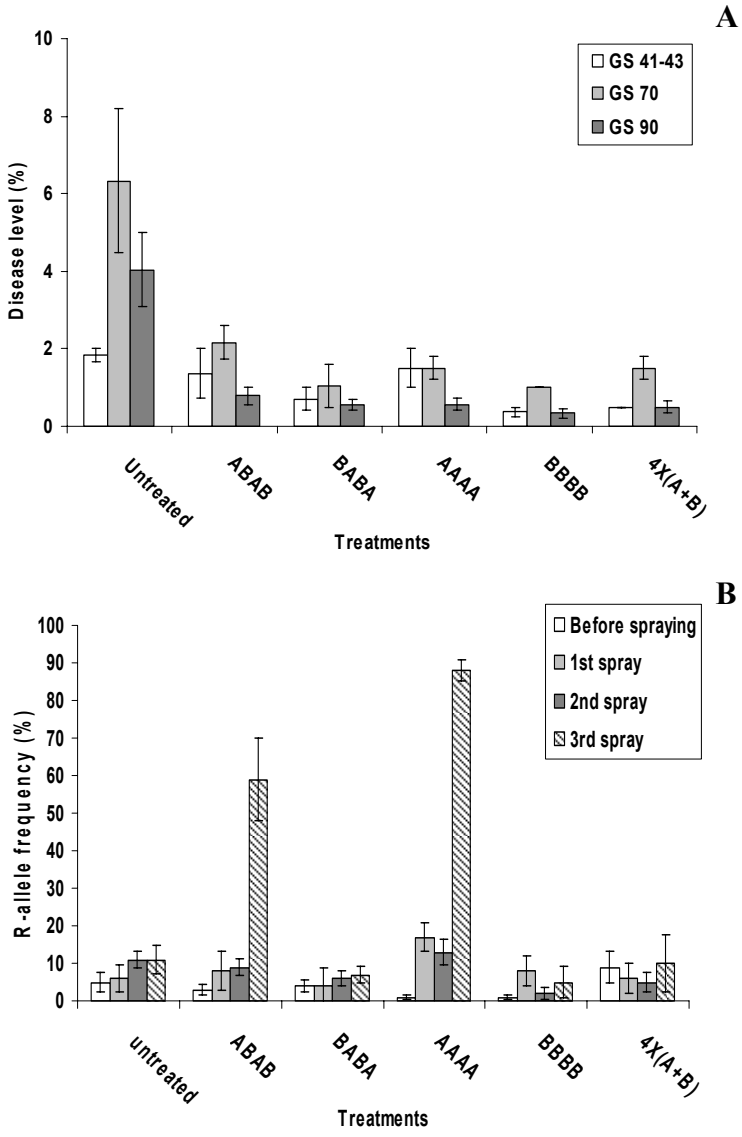


2.2.2 Effect of mixtures and alternation on QoI-resistance development

Location Inverness in 2002

Because of late establishment of mildew epidemics at Inverness, only three out of the four sprays for the mixture/alternation experiment were applied. Visual assessments showed that treated plots contained much less disease than the untreated plot at all sample timings (Figure 14A). Disease levels in the treated plot were similar, with least disease observed after four applications of Corbel. Highest R-allele frequencies were measured in plots treated with three sprays of Amistar, followed by plots treated with two Amistar sprays in the alternation programme with Corbel. Low levels of R-alleles were detected in populations that were exposed to three sprays of the Corbel/Amistar mixture (Figure 14B).

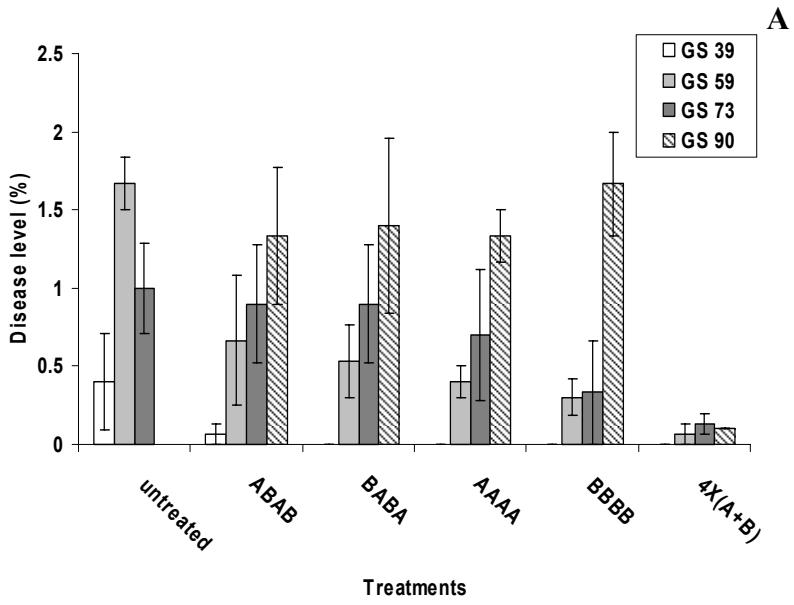
Figure 14. Effect of fungicide applications on disease levels and R-allele frequencies in powdery mildew populations at Inverness in 2002. Disease levels on Leaf 2 at GS 41-43 (after 1st spray), Leaf 2 at GS 70 (after 2nd spray) and Flag at GS 90 (after 3rd spray) (A) and R-allele frequencies after 1, 2 and 3 sprays of fungicides (B). A, Amistar at 0.5 l/ha; B, Corbel at 0.25 l/ha. Standard error bars are shown.

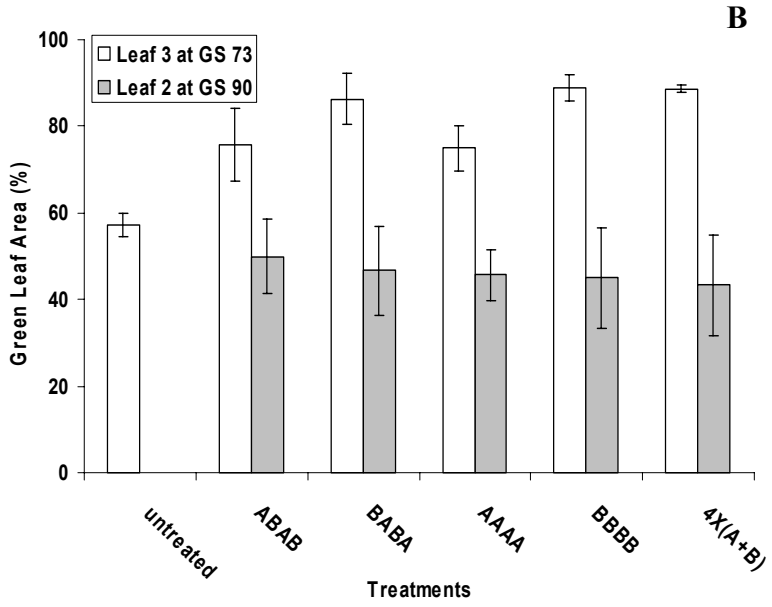


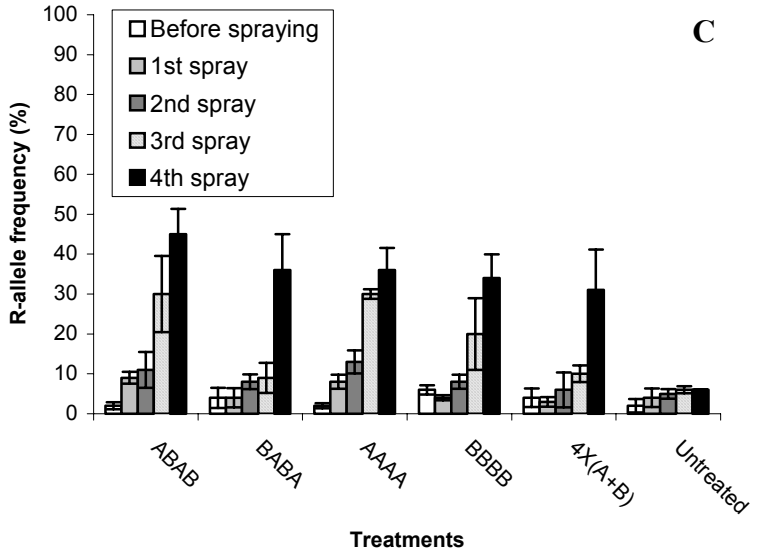
Location Terrington in 2002

Visual assessments showed that treated plots contained much less disease than the untreated plot after the first and second spray (Figure 15A). In comparison with other treatments, mixtures of Corbel and Amistar controlled mildew far better after three and four sprays. There were no significant differences between the different treatments with regard to Green Leaf Area of leaf 2 at GS 90 (Figure 15B). Final average R-allele frequencies between 30 and 45 % were measured for all treated plots including, unexpectedly, the Corbel-treated plot where no QoI-fungicides were applied (Figure 15C). After only three sprays the highest average R-allele frequencies (approximately 30 %) were measured in plots treated with three sprays of Amistar and plots that had received two sprays of Amistar in the alternation programme with Corbel. No significant increase in R-allele frequency was measured in mildew populations sampled from the untreated plots.

Figure 15. Effect of fungicide applications on disease levels, Green Leaf Area and R-allele frequencies in barley powdery mildew populations at Terrington in 2002. Disease levels on Leaf 3 at GS 39 (after 1st spray), Leaf 3 at GS 59 (after 2nd spray), Leaf 3 at GS 73 (after 3rd spray) and Leaf 2 at GS 90 (4th spray) (A), GLAof Leaf 3 (GS 73) and Leaf 2 (GS 90) (B) and R-allele frequencies after 1, 2, 3 and 4 sprays of fungicides (C). A, Amistar at 0.5 l/ha; B, Corbel at 0.25 l/ha. Standard error bars are shown; ND, visual disease and GLA assessments for untreated plots not done at GS 90.



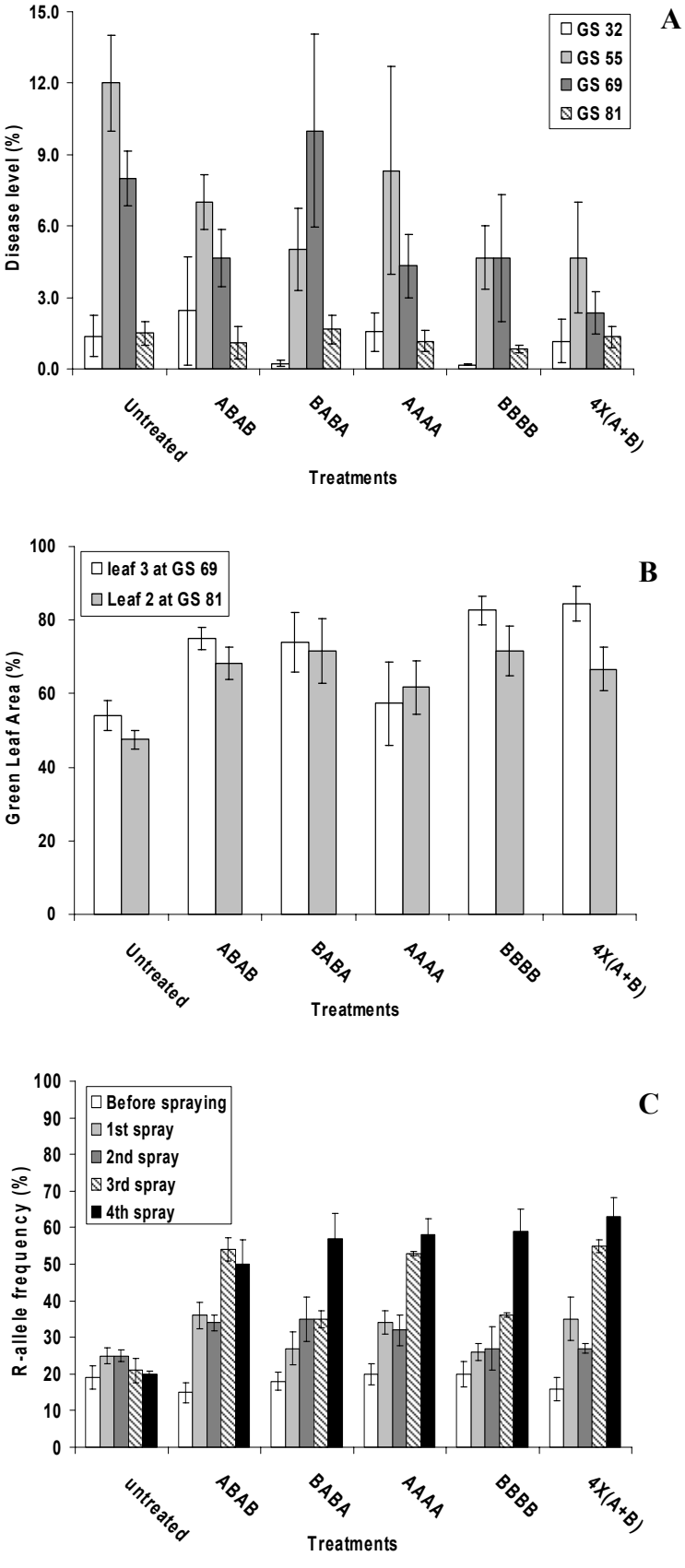




Location Terrington in 2003

Visual assessments showed treated plots generally contained much less disease than the untreated plot after the first, second and third sprays (Figure 16A). In comparison with other treatments, mixtures of Corbel and Amistar gave superior control of mildew after three sprays. The untreated plot had lower levels of GLA than treated plots (Figure 16B). In comparison with other treatments, four sprays of Amistar resulted in the lowest level of GLA. Final average R-allele frequencies between 50 and 63 % were measured for all treated plots, including the Corbel-treated plot (Figure 16C), whereas the R-allele frequency of the untreated plot remained low, between 19 and 25 % , during the season. After three sprays high R-allele frequencies above 50 % were measured for plots that had received two or more sprays containing Amistar.

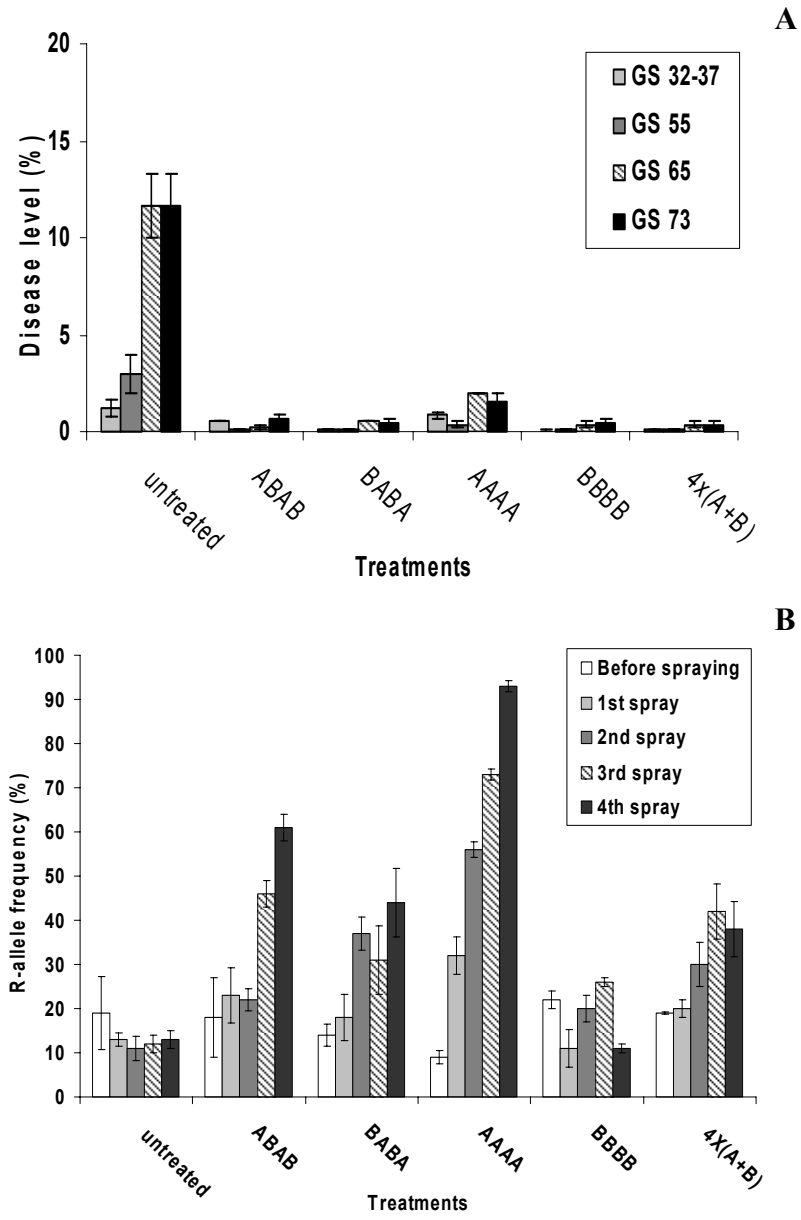
Figure 16. Effect of fungicides on disease levels, GLA and QoI resistance in powdery mildew populations at Terrington in 2003. Disease levels on Leaf 3 (L3) at GS 32 (after 1st spray), L3 at GS 55 (after 2nd spray), L3 at GS 69 (after 3rd spray) and L2 at GS 81 (4th spray) (A), GLA of L3 (GS 69) and 2 (GS 81) (B) and R-allele frequencies before and after fungicide sprays (C). A, Amistar at 0.5 l/ha; B, Corbel at 0.25 l/ha. Standard error bars are shown.



Location Edinburgh in 2003

Visual assessments showed excellent control of disease in all treated plots (Figure 17A). In comparison with other fungicide programmes, application of four sprays of Amistar was less effective in disease control. Highest increases in R-allele frequency, rising from 9 to 93% and 18 to 61 %, were measured after four sprays of Amistar, followed by alternation of Amistar/Corbel/Amistar/Corbel, respectively (Figure 17B). The R-allele frequency remained low, below 26 %, for both the untreated and Corbel-treated plots.

Figure 17. Effect of fungicide applications on disease levels and R-allele frequencies in barley powdery mildew populations sampled at location Edinburgh in 2003. Disease levels (percentage of leaf area covered with mildew pustules) on Leaf 3 at GS 32-37 (after first spray), GS 55 (after second spray), GS 65 (after third spray) and GS 73 (after fourth spray) (A) and R-allele frequencies before and after 1, 2, 3 and 4 sprays of fungicides (B). A, Amistar at 0.5 l/ha; B, Corbel at 0.25 l/ha. Standard error bars are shown.



2.2.3 Comparison between bioassays and PCR

Due to the amount of labour involved, fungicide sensitivity testing was only done on powdery mildew present on leaves sampled from plots treated with three sprays of Amistar at 0.33 l/ha during the 2002/03 growing season. An overview of the test results is given in Table 7.

Location Terrington

Samples taken before fungicide applications showed an average of 6.5% QoI-resistant conidia. The average proportion of QoI-resistant conidia rose to 18.3, 33 and 44 % after the first, second and third sprays of Amistar, respectively. This indicates that consecutive sprays of QoI fungicide exerted selection pressure that resulted in an increase in the frequency of resistance in the pathogen population. No samples were taken after the fourth spray because all mildew observed in the plots had already entered the cleistothecial stage of development. PCR results showed a similar trend with average R-allele frequencies of 5.7, 7.3, 8.0 and 31.0 % before spraying and after the first, second and third spray respectively. Compared to the bioassay, the lower frequency after the first two sprays might be due to a small population size, low viability of QoI-sensitive conidia and/or detection of S-alleles in DNA from dead conidia.

Location Inverness

Samples taken before fungicide applications showed an average of 6.8% QoI-resistant conidia. The frequencies of R conidia in the three replicate plots varied between 0.5 and 13.0 %, with an average of 6.8 %. An average R-allele frequency of 2.3 % was measured with PCR. Unfortunately, most samples received after the first fungicide application had been in transit for two days and did not produce any new spores. Only four viable mildew pustules from one of the replicate plots could be transferred to fresh treated and untreated leaf segments ("individual pustule test"). From these, conidia from one of the pustules grew on both untreated segments (controls) and on segments treated with 50 ppm azoxystrobin. A repeat sample taken after the first fungicide application was in transit for four days and no viable pustules were found after incubation. Because of the transport problems no further samples were taken from this location.

Location Edinburgh

Samples from this site were also affected by delays (up to seven days in transit) and high temperatures unfavourable for sporulation. Samples taken after the third spray arrived after two days in transit and from two of the replicate plots it was possible to carry out the "individual pustule test" from 2 and 3 pustules respectively. None of these produced growth on leaf segments treated with 50 ppm azoxystrobin.

Table 7. Results of barley powdery mildew sensitivity testing during the 2002/03 growing season.

Trial Site - ADAS Terrington - England

Sample date		Repetition 1		Repetition 2		Repetition 3	
		untreated segments	50ppm azoxystrobin segments	untreated segments	50ppm azoxystrobin segments	untreated segments	50ppm azoxystrobin segments
15/05/2002 (Pre-application)	No.of segments infected	25 / 25	24 / 25	none of the pustules on the sample material from this plot were viable		25 / 25	25 / 25
	Inoc. Density (spores/seg)*	956	956			898	898
	Total no.of pustules	2433	130			2418	189
	% of resistant pustules	-	5%			-	8%
28/05/2002 (after 1st spray)	No.of segments infected	25 / 25	24 / 25	25 / 25	25 / 25	25 / 25	25 / 25
	Inoc. Density (spores/seg)	824	824	720	720	656	656
	Total no.of pustules	1878	279	2253	406	1503	324
	% of resistant pustules	-	15%	-	18%	-	22%
11/06/2002 (after 2nd spray)	No.of segments infected	25 / 25	25 / 25	none of the pustules on the sample material from this plot were viable		25 / 25	25 / 25
	Inoc. Density (spores/seg)	410	410			192	192
	Total no.of pustules	1178	531			767	160
	% of resistant pustules	-	45%			-	21%
25/06/2002 (after 3rd spray)	No.of segments infected	25 / 25	20 / 25	25 / 25	25 / 25	none of the pustules on the sample material from this plot were viable	
	Inoc. Density (spores/seg)	22	22	138	138		
	Total no.of pustules	148	39	505	270		
	% of resistant pustules	-	33%	-	55%		

* calculated by counting the number of conidia/cm² and multiplying by the area of the leaf segment

Trial Site - Findon Mains - Scotland

Sample date		Repetition 1		Repetition 2		Repetition 3		Repetition 4	
		untreated segments	50ppm azoxystrobin segments	untreated segments	50ppm azoxystrobin segments	untreated segments	50ppm azoxystrobin segments	untreated segments	50ppm azoxystrobin segments
05.06.2002 (Pre-application)	No.of segments infected	25 / 25	24 / 25	25 / 25	25 / 25	none of the pustules on the sample material from this plot were viable		25 / 25	4 / 25
	Inoc. Density (spores/seg)	338	338	340	340			464	464
	Total no.of pustules	1252	85	1010	135			1096	5
	% of resistant pustules	-	7%	-	13%			-	0,5%
13.06.2002 (after 1st spray)	No.of segments infected			none of the pustules on the sample material from this plot were viable		none of the pustules on the sample material from this plot were viable		(individual pustule test)	
	Inoc. Density (spores/seg)								
	Total no.of pustules							4	1
	% of resistant pustules								
20.06.2002 (after 1st spray) repetition	No.of segments infected	none of the pustules on the sample material from this plot were viable		none of the pustules on the sample material from this plot were viable		none of the pustules on the sample material from this plot were viable		none of the pustules on the sample material from this plot were viable	
	Inoc. Density (spores/seg)								
	Total no.of pustules								
	% of resistant pustules								

* calculated by counting the number of conidia/cm² and multiplying by the area of the leaf segment

Trial Site - Boghall Bush - Scotland

Sample date		Repetition 1		Repetition 2		Repetition 3		Repetition 4	
		untreated segments	50ppm azoxystrobin segments	untreated segments	50ppm azoxystrobin segments	untreated segments	50ppm azoxystrobin segments	untreated segments	50ppm azoxystrobin segments
25.06.2002 (Pre-application)	No.of segments infected	none of the pustules on the sample material from this plot were viable		none of the pustules on the sample material from this plot were viable		none of the pustules on the sample material from this plot were viable		none of the pustules on the sample material from this plot were viable	
	Inoc. Density (spores/seg)								
	Total no.of pustules								
	% of resistant pustules								
01.07.2002 (after 1st spray)	No.of segments infected	none of the pustules on the sample material from this plot were viable		none of the pustules on the sample material from this plot were viable		none of the pustules on the sample material from this plot were viable		none of the pustules on the sample material from this plot were viable	
	Inoc. Density (spores/seg)								
	Total no.of pustules								
	% of resistant pustules								
08.07.2002 (after 2nd spray)	No.of segments infected	none of the pustules on the sample material from this plot were viable		none of the pustules on the sample material from this plot were viable		none of the pustules on the sample material from this plot were viable		none of the pustules on the sample material from this plot were viable	
	Inoc. Density (spores/seg)								
	Total no.of pustules								
	% of resistant pustules								
22.07.2002 (after 3rd spray)	No.of segments infected	(individual pustule test)		(individual pustule test)		none of the pustules on the sample material from this plot were viable		none of the pustules on the sample material from this plot were viable	
	Inoc. Density (spores/seg)								
	Total no.of pustules	2	0	3	0				
	% of resistant pustules								

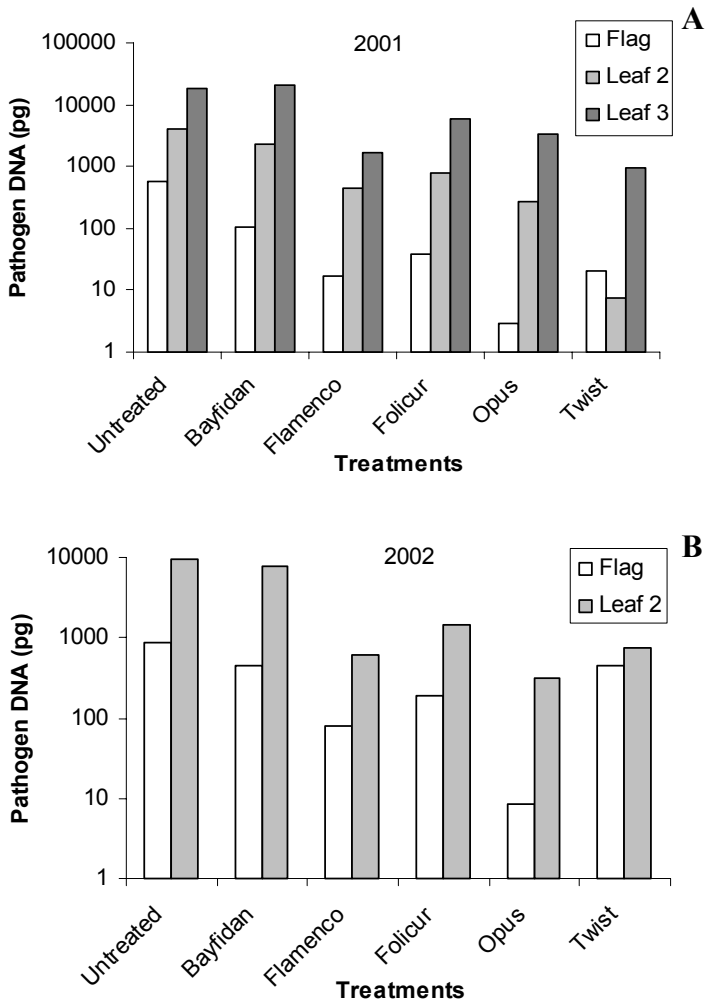
* calculated by counting the number of conidia/cm² and multiplying by the area of the leaf segment

2.3 Septoria field experiments at Rothamsted

2.3.1 Field trials in 2001 and 2002

Disease severity on leaf samples collected in 2002 ranged from 0.1 to 46 %, recorded for flag leaves from the Opus (epoxiconazole) treated plot and leaf 2 from the untreated plot, respectively. The 0.1 % infected leaf sample represented a single lesion from ten leaves pooled together and contained approximately 10 pg of *Septoria tritici* DNA, whereas approximately 10 ng was detected in the most severely diseased leaves (Figure 18). Even in samples without visible symptoms, e.g. flag leaves of Opus (epoxiconazole) and Twist (trifloxystrobin) treated plots in 2001, pathogen DNA could still be detected. In both years, Bayfidan (triadimenol) sprays were not able to control leaf blotch, as similar disease levels were measured in the untreated plots. Opus was the most active triazole for both years while the efficacy of Twist (trifloxystrobin), the most effective fungicide in 2001, was slightly reduced in 2002 in comparison with the other treatments.

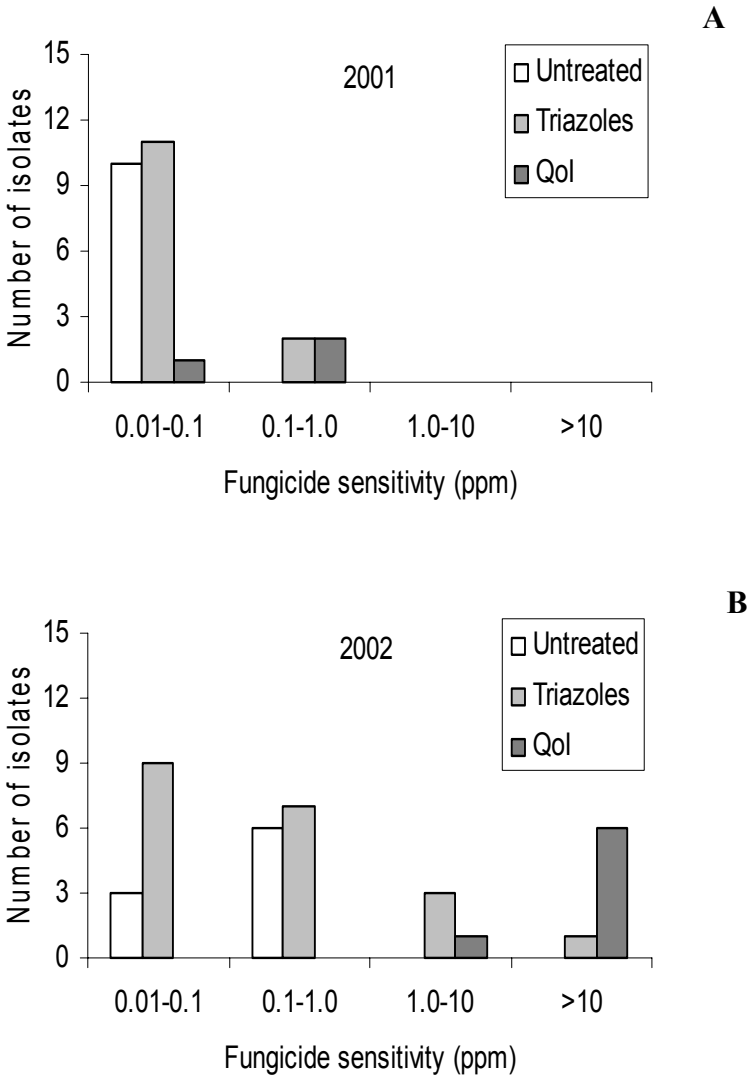
Figure 18. Efficacy of different fungicide treatments to control Septoria leaf blotch. Plots untreated or treated with three sprays of Bayfidan (0.33 l/ha), Flamenco (0.83 l/ha), Folicur (0.66 l/ha), Opus (0.66 l/ha) or Twist (1.33 l/ha). Disease levels for Riband in 2001 (A) and cv. Savannah in 2002 (B).



Fungicide sensitivity testing and characterisation of strains isolated from trial plots

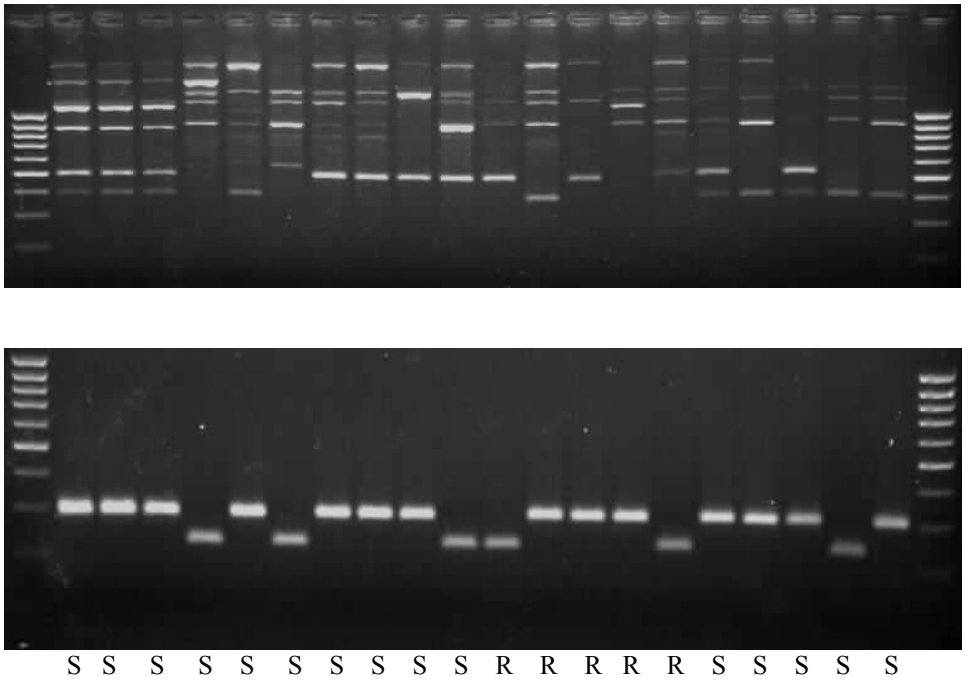
A total of 62 strains were isolated, and ED_{50} values for azoxystrobin determined *in vitro* using OD measurements (Figure 19).

Figure 19. Azoxystrobin sensitivity testing of *Septoria tritici* isolates. A, strains isolated from fungicide treated and untreated plots of cultivar Riband in 2001; B, strains isolated from plots of cultivar Savannah in 2002.



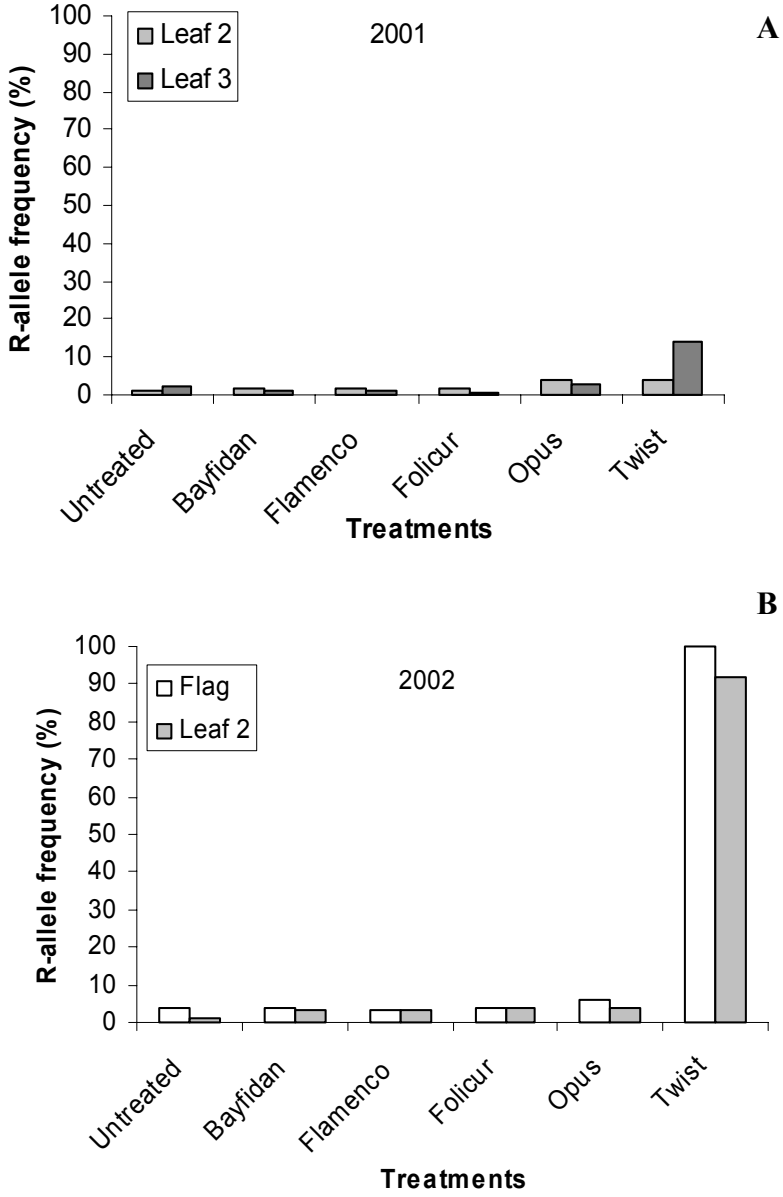
Sequencing and allele-specific real-time PCR tests showed that the 11 most resistant strains (ED_{50} values >1.0 μg ml^{-1}), all isolated in 2002, carried only resistant (A143) alleles. No resistant strains were isolated in 2001, whereas all strains (n = 7) isolated from the Twist (trifloxystrobin) treated plot in 2002 were resistant. The remaining four resistant strains were isolated from Bayfidan (triadimenol) (n = 1), Flamenco (fluquinconazole) (n = 1) and Folicur (tebuconazole) (n = 2) treated plots. Only sensitive (G143) alleles were detected in sensitive isolates. Further characterisation of resistant strains showed a non-clonal origin and both mating types could be detected (Figure 20).

Figure 20. RAPD profiling and mating type analysis of *S. tritici* isolates. Top panel, RAPD patterns; bottom, mating type products of 221 and 290 bp for mating types 1-1 and 1-2, respectively. Lanes 1 to 5, QoI sensitive isolates from Twist treated plots in 2001; 6-10, QoI sensitive isolates from untreated plots in 2001; 11-14, QoI resistant isolates from Twist treated plots in 2002; 15, QoI resistant isolate from Folicur treated plot; 16-20, QoI sensitive isolates from untreated plots in 2002.



Retrospective PCR testing of stored leaf samples confirmed the results of isolate sensitivity testing. Due to a detection threshold of approximately 2 %, only samples with R-allele frequencies higher then 3 % were regarded as true positives., High R-allele frequencies between 90 and 100 % were detected in populations sampled from the Twist (trifloxystrobin) treated plot in 2002, whereas low levels of R-alleles between 3 and 5% were detected in the other leaf population samples with the exception of leaf 2 from the untreated plot (Figure 21). Interestingly, R-alleles were also detected in the 2001 samples, particularly in the leaf 3 population of the Twist treated plot, where a relatively high frequency of 14 % was measured. A frequency of 4 % was detected in leaf 2 populations from the Opus- and Twist treated plot whereas frequencies < 3 % were detected in leaf populations from the untreated plot and remaining leaf population samples from treated plots.

Figure 21. Effect of different fungicide applications on the development of QoI-resistance using G143A as marker. Control, untreated plot; other plots treated with three sprays of Bayfidan (0.33 l/ha), Flamenco (0.83 l/ha), Folicur (0.66 l/ha), Opus (0.66 l/ha) or Twist (1.33 l/ha). A, R-allele frequencies in leaf populations of cv. Riband sampled in 2001; B, R-allele frequencies in leaf populations of cv. Savannah in 2002.



2.3.2 Role of ascospores in further spread of R-alleles during 2003

At the start of the season (22 January 2003), 100 leaves showing pycnidia-bearing lesions were sampled randomly from the field. In total, 94 isolates of *S. tritici* were isolated. Four isolates, one carrying A143 alleles, did not sporulate on agar and were unable to grow in liquid culture. In total, 29 out of the remaining 90 isolates grew well in media amended with 1.0 μg ml^{-1} trifloxystrobin. For all these isolates, the presence of A143 (R-) alleles was confirmed by real-time PCR. When leaf populations (50 leaves with symptoms) from the SW and NE plots were tested directly by PCR, similar R-allele frequencies were measured; 30 and 32%, respectively. After fungicide applications, the disease was well controlled in plots surrounding the

squares, and visible symptoms were absent in the top three leaf layers before 2 July. Real-time PCR testing of leaf samples (25 leaves sampled randomly) confirmed different disease levels in the untreated and QoI-treated squares (Table 8).

Table 8. Leaf blotch infection levels and R-allele (A143) frequencies in leaf populations of *Septoria tritici* sampled on 2 July 2003.

Area/treatment	Leaf layer	Disease level[1]	R-allele frequency (%)
Untreated square	Flag	90 ± 28	68 ± 3
	Leaf 2	423 ± 48	30 ± 4
	Leaf 3	11,910 ± 710	44 ± 2
QoI-treated square	Flag	46 ± 24	97 ± 2
	Leaf 2	321 ± 16	97 ± 0
	Leaf 3	2,755 ± 79	96 ± 1

[1]Amount of pathogen DNA in pg per 50 ng of total sample DNA. Mean values ± standard errors are given.

A clear progression of disease from the lower leaves to the upper canopy was observed in both squares. Disease levels were lower in the treated square, but higher R-allele frequencies were detected. After development of flag leaf symptoms in plots surrounding the squares, 25 flag leaves per sampling point of one m^2 were collected at various positions from the mid point of each side of the squares on 17 July (Table 9).

Table 9. Frequency of R-alleles (A143) in flag leaf populations of *Septoria tritici* on 17 July 2003.

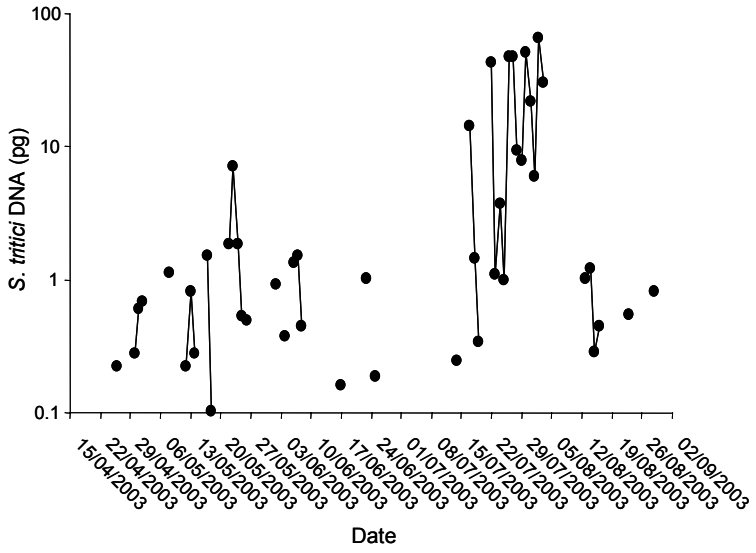
		R-allele frequency (%) at different sampling points[1]							
		NE		SE		SW		NW	
Square	Inside	5 m	25 m	5 m	25 m	5 m	25 m	5 m	25 m
Untreated	37 ± 2	31 ± 1	20 ± 6	33 ± 2	14 ± 2	51 ± 0	50 ± 1	49 ± 1	34 ± 2
QoI treated	99 ± 0	97 ± 2	64 ± 1	71 ± 2	45 ± 0	79 ± 1	62 ± 0	99 ± 0	7 ± 1

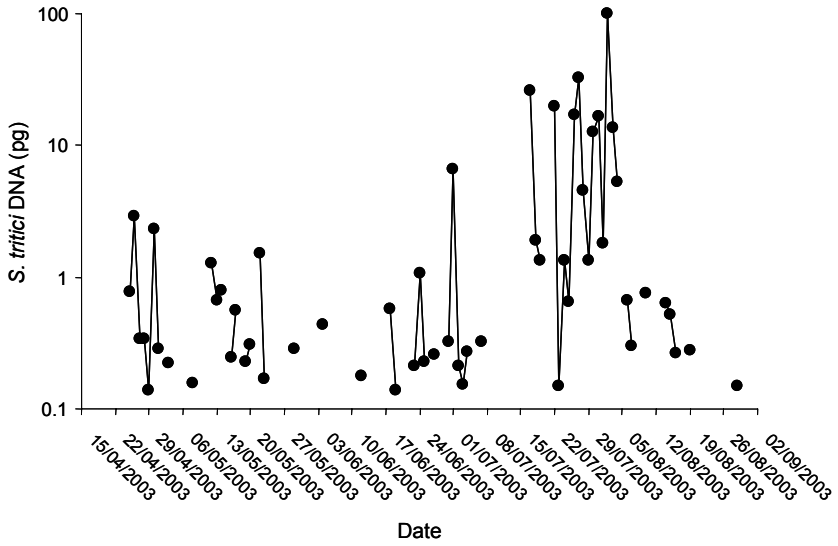
[1]Flag leaves with lesions were sampled within the squares and at 5 and 25 m distance from these squares in four different directions (North East, South East, South West and North West). Mean values ± standard errors are given.

In comparison to samples collected at 25 m distance from the QoI-treated squares, the frequency of R-alleles was higher at 5 m. At 25 m distance, the lowest R-allele frequency (7%) was measured in the North West (NW) direction, where lower infection levels on flag leaves were also observed (results not shown). In the untreated plot, highest R-allele frequencies (50 and 51%) were measured in the South West direction, which is equivalent to North East for the QoI-treated square.

Dispersal of ascospores and incidence of G143A. From 15 April until 3 September, a month after harvest, the amount of *S. tritici* spores in air samples was measured by real-time PCR (Figure 22). The presence of ascospores, identified by size and shape, was confirmed by microscopy. Larger, asexual conidia were not detected. As microscopy was laborious and at times unreliable, due to the presence of many other fungal spores of similar morphology, masking by pigmented spores and problems in focussing because of an uneven coating layer, only a sub set of samples (n = 14) was analysed and no linear correlation with PCR was found ($R^2 = 0.12$).

Figure 22. Temporal dispersal of ascospores measured in the centre of the untreated (A) and QoI-treated square (A). The amount of *S. tritici* DNA (pg) per daily aerosol tape sample is presented; lines connect consecutive data points. In the QoI-treated plot, no aerosol samples were available from 7 - 14 June 2003 due to a faulty spore trap motor.

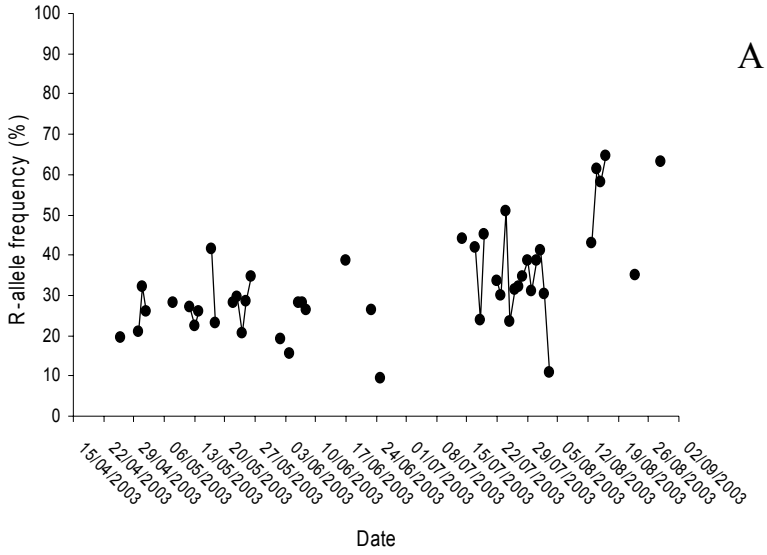


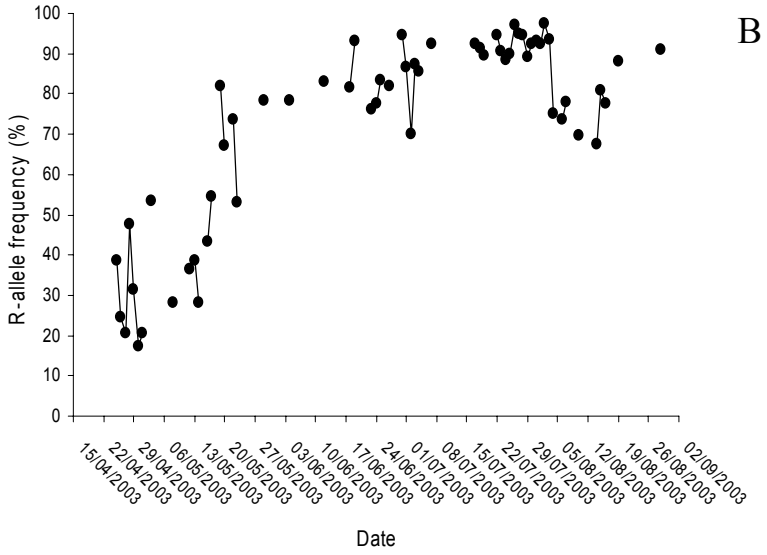


B

Ascospore dispersal patterns were similar for both the untreated and QoI-treated squares (Figure 22). Relatively low levels of ascospores were trapped before mid-July until mid-August, the peak of ascospore production. The amount of ascospores trapped decreased after 3 August, the day of harvest, most likely due to removal of pseudothecia present in the standing straw. Fungicide treatment had an immediate affect on R-allele frequency (Figure 23).

Figure 23. Temporal dynamics of QoI-resistant alleles present in aerosol populations of ascospores sampled in the centre of the untreated (A) and QoI-treated square (B). Lines connect consecutive data points. For the QoI-treated plot, no aerosol samples were available from 7-14 June 2003 due to a faulty spore trap motor.

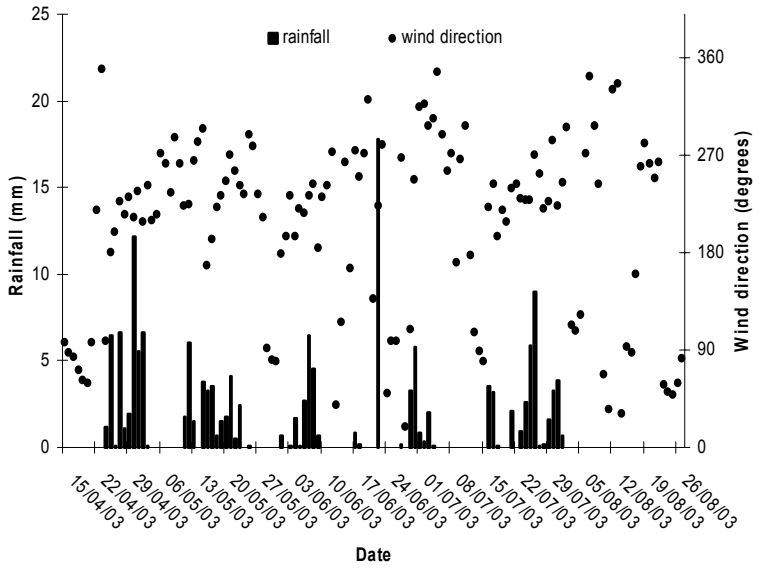




After the first QoI application, the R-allele frequency increased rapidly from 35 to 80% in the QoI-treated square. After the second spray, R-allele frequencies up to 95% were measured, while after harvest a slight drop in R-allele frequency was observed (Figure 23B). Ascospores trapped in the untreated square showed

the opposite trend; no significant shift in R-allele frequency was detected before harvest, with an average R-allele frequency of 35%, whereas after harvest R-higher R-allele frequencies up to 60% were measured (Figure 23B). There was no clear correlation between ascospore production and rainfall events (Figure 24).

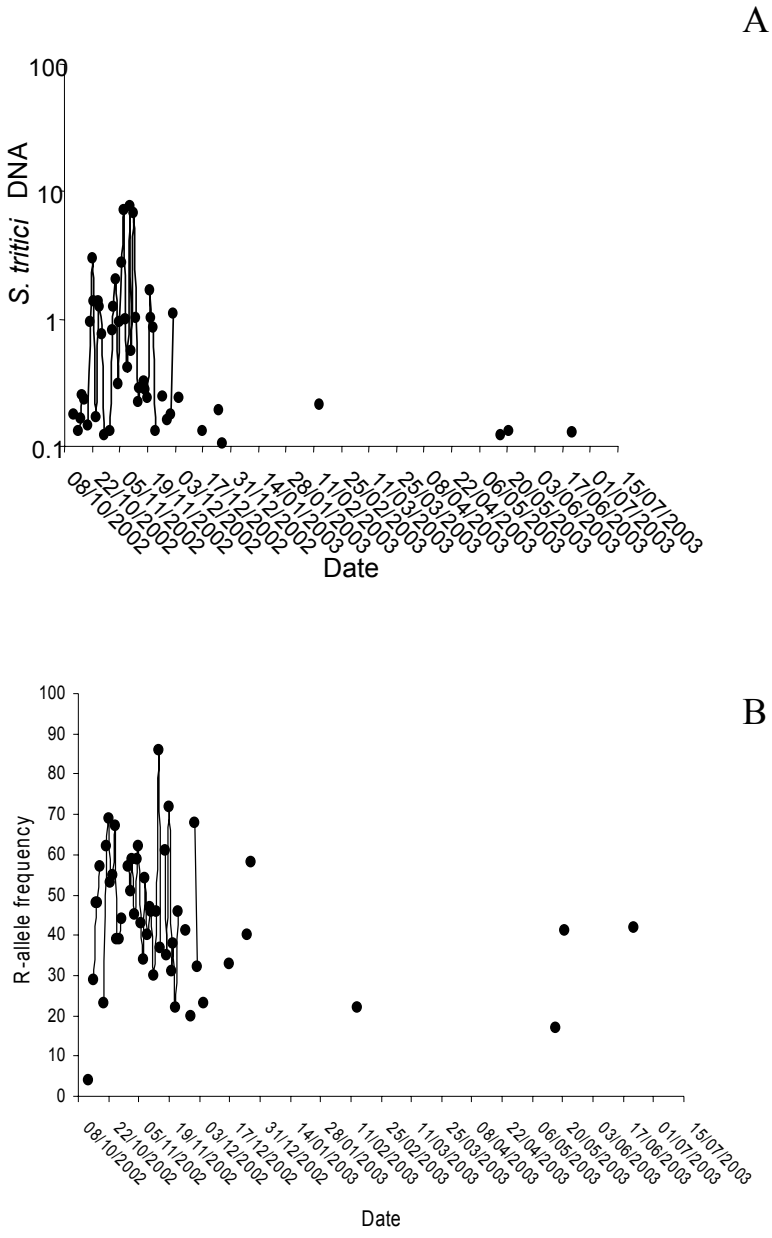
Figure 24. Rainfall and daily mean wind direction data recorded during the period of aerosol sampling. Wind direction is the mean of the previous 24 hours up to 9 am. Wind direction 0 and 360 degrees is equivalent to North.



Additional analysis of spore tapes obtained from a trap operated in a winter barley field from 8 October 2002 until 14 July 2003 showed that *S. tritici* ascospores were present almost every day, at concentrations between 5 and 200 ascospores m^{-3}, until the first week of December (Figure 25A). Between January and mid-July, ascospores were only detected four times, at concentrations between three and seven ascospores m^{-3}. This pattern of ascospore dispersal confirms the importance of overwintering pseudothecia on stubble as a primary inoculum source for newly emerging wheat crops, and the subsequent exhaustion of this source during winter.

Fungicide sensitivity tests and PCR assays showed that all azoxystrobin-resistant isolates tested, with EC_{50} >1.0 μg ml^{-1}, carried A143 cytochrome *b* alleles. Using cytochrome *b* alleles as a target, real-time PCR could detect as little as 0.01 pg of genomic DNA of *S. tritici*. Assuming a single spore contains at least one hundred mitochondria, the detection threshold of 0.1 pg of genomic DNA that was used in the spore trapping analysis to simultaneously detect R-allele frequencies would correspond with approximately three ascospores per m^{-3} air sample. From mid-April until mid-July, the ascospore concentration rarely exceeded 30 ascospores m^{-3}, whereas during the peak of ascospore production, measured during the end of July and early August, almost 100-fold more ascospores were measured (Figure 22).

Figure 25. Temporal dispersal of *S. tritici* ascospores carrying G143A cytochrome *b* alleles in a barley field. The distance to the nearest wheat crop grown in the previous season was 30 m. Lines connect consecutive data points. A; amount of ascospores as measured by pathogen DNA (pg) per daily aerosol tape sample; B: R-allele frequencies for trapped ascospore populations. Samples were not available during 9 – 15 January.



When analysing the G143A allele distribution in ascospore air samples, the daily frequencies measured for the barley field varied from 4 to 86 %, with an average of 46 % (Figure 25B). This variation is probably due to the relatively low numbers of ascospores caught on tapes, migrating from fields where different fungicide application strategies had been used. It has been shown that consecutive treatments with QoIs can rapidly increase the proportion of resistant strains within populations through selection and subsequent asexual multiplication within a single season. For *S. tritici* conidia, only limited spread over a few metres is expected, consistent with other studies of splash-dispersed arable crop pathogens. Our data show that ascospores were

dispersed over longer distances, as higher R-allele frequencies, carried by ascospores, were measured in *S. tritici* leaf populations sampled at 5 and 25 m distance from the QoI-treated square than around the untreated square. Based on an estimated latent period of up to 300 day degrees, the lesions observed on flag leaves sampled on 17 July must have developed from ascospores dispersed from 30 June till 3 July. During this period, the wind changed from North to East and then later predominantly from a South West to North West direction (Figure 24). This agrees well with the long-distance (25 m) spread of R-alleles measured for lesions sampled in the North East, South East, and, to a lesser extent, South West direction from the QoI-treated square (Table 9). The relatively high frequencies of 50 and 51% measured in the South West direction of the untreated square, at 5 and 25 m distance, respectively, were probably caused by dispersal of ascospores in the North East direction from the QoI-treated square, travelling distances of 85 and 65 m, respectively. In particular, the large amount of spores dispersed on 30 June, mostly caught on tape on 1 July (Figure 22), could have blown in this direction (Figure 24). After harvest, fewer ascospores were trapped and R-allele frequencies in these populations fluctuated more (Figure 23). This variation can be explained by ascospore arrival from different inoculum sources. For example, relatively high and low R-allele frequencies were measured for ascospores captured above the untreated and QoI-treated square during 6 until 16 August (Figure 23). Based on the wind direction during this period, mainly West to East (via North) (Figure 24), these ascospores were not dispersed from the QoI-treated square and, based on the high R-allele frequencies, must have originated from other fungicide-treated wheat fields nearby.

2.4 Septoria QoI resistance survey

2.4.1 PCR testing of field populations using G143A as marker

After the discovery of QoI-resistant strains of *S. tritici* in late summer 2002, an intensive survey was conducted during spring and late summer 2003 in order to establish the geographical extent of resistance before and after fungicide sprays. Septoria leaf blotch samples (50 randomly sampled green leaves with clear Septoria lesions) were collected by individual farmers, ADAS consultants and Agrochem industry partners and send to Rothamsted Research for testing. The samples listed in Table 10 were mostly collected from commercial fields before and after application of fungicides. Most fields had received two sprays with mixtures of azoles and QoIs during the season.

Table 10. Overview of samples received during spring and summer in 2003.

Sample number	Location	Cultivar	Sample timing	Number of QoI-based sprays	Real-time PCR R-allele frequency (%)
1a	Woodfield Farm, Renhold, Beds	Claire	Spring	0	24
1b	Woodfield Farm, Renhold, Beds	Claire	Summer	2	100
2	Long Ashton Research Station, Somerset	Claire	Spring	0	26
3	Herne Manor Farm, Toddington, Dunstable, Beds	Soissons	Spring	0	11
4a	Manor Farm, Bugbrook, N. Hants	Hereward	Spring	0	34
4b	Manor Farm, Bugbrook, N. Hants	Hereward	Summer	2	100
5	Seawall Grounds, Foxley, Towcester, N. Hants	Malacca	Spring	0	35
6	Heath Farm, Farthingstone, Towcester, N. Hants	Malacca	Spring	0	39
7a	Burman Farms, Hartwell Rd. Roade, N. Hants	Richmond	Spring	0	45
7b	Burman Farms, Hartwell Rd. Roade, N. Hants	Richmond	Summer	2	84
8a	Lomas Farms, Stapenhill, Burton-on-Trent, Staffs	Consort	Spring	0	26
8b	Lomas Farms, Stapenhill, Burton-on-Trent, Staffs	Consort	Summer	2	89
9	Manor Farm, Clipstone, Leighton Buzzard, Beds	Malacca	Spring	0	31
10	Saxby Farms, Irchester, N. Hants	Malacca	Spring	0	41
11	College Farm, Woodend, Nr. Towcester, N. Hants	Claire	Spring	0	33
12	Ryehill Farm, Woodford Halse, Daventry, N. Hants	Claire	Spring	0	22
13a	LittleKetton Farm, Coatham Mundeville, Co Durham	Tanker	Spring	0	7
13b	LittleKetton Farm, Coatham Mundeville, Co Durham	Tanker	Summer	2	99
14	Manor Farm, Garford, Abingdon, Oxon	Consort	Spring	0	73
15	Essington, Wolverhampton,	Consort	Spring	0	20
16	Coven, Wolverhampton,	Claire	Spring	0	10
17	Shrewsbury, Shrops	Consort	Spring	0	14
18	Denham Grange, Malton, N. Yorks	Consort	Spring	0	44
19	Brewood, Staffs	Claire	Spring	0	14
20a	Hall Farm, Newton-on-Trent, Lincoln	Equinox	Spring	0	10
20b	Hall Farm, Newton-on-Trent, Lincoln	Equinox	Summer	2	93
21a	Hall Farm, Newton-on-Trent, Lincoln	Claire	Spring	0	17
21b	Hall Farm, Newton-on-Trent, Lincoln	Claire	Summer	2	48
23	Netherhampton farm, Netherhampton, Salisbury,Wilts	?	Spring	0	60
24	Fenn Lane Farm, Nuneaton, Warks	Claire	Spring	0	25
25a	Newham Hall, Newby, Middlesborough	Consort	Spring	0	11
25b	Newham Hall, Newby, Middlesborough	Consort	Summer	3	69
26	Barton Farm, Sherborne, Dorset	Option	Spring	0	19

27	Stonards Farm, High Wych, Sawbridgeworth, Herts.	Savannah	Summer	0	12
28a	Home Farm, Chichley, Newport Bagnell, Bucks	Consort	Spring	0	26
28b	Home Farm, Chichley, Newport Bagnell, Bucks	Consort	Summer	3	88
29	Carlow, Ireland	Madrigal	Spring	0	52
30	Denfind, Newbigging, Dundee	Consort	Spring	0	16
31	Cunmont Farm, Newbigging, Dundee	Consort	Spring	0	12
32	Manor Farm, Byfield, Nr. Daventry, Northants	Consort	Spring	0	36
33a	Uplyme House, Orchard Lane, Harrold, Beds	Option	Spring	0	23
33b	Uplyme House, Orchard Lane, Harrold, Beds	Option	Summer	2	97
34a	Walthambury farm, Great Waltham, Chelmsford, Essex	Consort	Spring	0	19
34b	Walthambury farm, Great Waltham, Chelmsford, Essex	Consort	Summer	2	95
35	Manor Farm, Tingsith, Milton Keynes, Beds	Consort	Spring	0	26
36	Vicarage Farm, East Chaldon, Dorchester, Dorset	Savannah	Spring	0	10
37a	Lovett, Measham, Leics	Consort	Spring	0	29
37b	Lovett, Measham, Leics	Consort	Summer	2	95
38a	Taylors, Ingleberry, Shepsted, Loughbrough, Leics	Claire	Spring	0	20
38b	Taylors, Ingleberry, Shepsted, Loughbrough, Leics	Claire	Summer	1	77
39	Hall Farm, Irthlingborough, Northhants	Consort	Spring	0	32
40	Streatley Farm, Streatley, Reading, Berks	Claire	Spring	0	39
41a	Sescut Farm Woodeaton, Oxon	Solstice	Spring	0	41
41b	Sescut Farm Woodeaton, Oxon	Solstice	Summer	3	93
42a	Walthambury Farm, Great Waltham, Chelmsford	Consort	Spring	0	17
42b	Walthambury Farm, Great Waltham, Chelmsford	Consort	Summer	2	88
43	Hitchin, Beds	Malacca	Spring	0	33
44a	Manor Farm, Threekingham, Sleaford, Lincs	Tanker	Spring	0	19
44b	Manor Farm, Threekingham, Sleaford, Lincs	Tanker	Summer	+?	60
45a	Eau Farm, (Swaton) Ltd, Sleaford, Lincs	Consort	Spring	0	24
45b	Eau Farm, (Swaton) Ltd, Sleaford. Lincs	Consort	Summer	?	84
46a	Naby Farm, Hanby, Grantham, Lincs	Tanker	Spring	0	17
46b	Naby Farm, Hanby, Grantham, Lincs	Tanker	Summer	+?	93
47	Ravleby Farms, South Ravleby, Sleaford, Lincs	Solstice	Spring	0	30
48a	North End Farm, Swaton, Sleaford, Lincs	Richmond	Spring	0	22
48b	North End Farm, Swaton, Sleaford, Lincs	Richmond	Summer	+?	82
49a	Manor Farmhouse, Stanion, Kettering Northants	Consort	Spring	0	25
49b	Manor Farmhouse, Stanion, Kettering Northants	Consort	Summer	2	99
50	New Buildings Farm, Harrold, Beds	Option	Spring	0	23
51a	Hill Farm, Wingfield, Leighton Buzzard, Beds	Hereward	Spring	0	45
51b	Hill Farm, Wingfield, Leighton Buzzard, Beds	Hereward	Summer	2	99
52b	Burman Farms, Roade, Northants	Tanker	Spring	0	40
52b	Burman Farms, Roade, Northants	Tanker	Summer	2	91
53	Moorfield Lodge, Orlingbury, Northants	Savannah	Spring	0	46
54	Manor Farm, Foxley, Towcester, Northants	Consort	Spring	0	34
55a	South West Farm, Canons Ashby, Daventry	Claire	Spring	0	32
55b	South West Farm, Canons Ashby, Daventry	Claire	Summer	2	89
56a	Home Farm, Haynes, Beds	Tanker	Spring	0	10
56b	Home Farm, Haynes, Beds	Tanker	Summer	2	93
57	Bassmead Manor, Staploe, Cambs	Tanker	Spring	0	69
58a	Brook Farm, Swineshead, Beds	Malacca	Spring	0	26
58b	Brook Farm, Swineshead, Beds	Malacca	Summer	2	97
59	Lyndon Mall Farm, Lyndon Oakham, Rutland	Tanker	Spring	0	24
60	Morley Research Centre, Wymondham, Norfolk	Access	Spring	0	18
61a	Rothamsted Research, Harpenden, Herts	Savannah	Spring	0	38

61b	Rothamsted Research, Harpenden, Herts	Savannah	Summer	2	97
61c	Rothamsted Research, Harpenden, Herts	Savannah	Summer	0	37
62a	Heathersett, Norfolk	Consort	Spring	0	38
62b	Heathersett, Norfolk	Consort	Summer	2	94
62c	Heathersett, Norfolk	Consort	Summer	0	48
63a	Kent	Consort	Spring	0	88
63b	Kent	Consort	Summer	2	100
64	Awards, Lincolnshire	Tanker	Spring	0	31
65	Metheringham, Lincolnshire	Claire	Spring	0	42
66a	Waterford, Ireland	Claire	Spring	0	7
66b	Waterford, Ireland	Claire	Summer	2	83
66c	Waterford, Ireland	Claire	Summer	0	7
67	Abernethy, Scotland	Riband	Spring	0	<3
68	Yealmpton, Devon	Reaper	Spring	0	10
69	Chillington, Devon	Equinox	Spring	0	<3
70	Calais, France	?	Spring	0	<3

PCR monitoring for the presence of G143A in fungal leaf populations revealed that QoI resistance was widespread in the UK during spring 2003 (Table 10 & Figure 26). Out of the 70 fields investigated, only two fields in Devon and Scotland tested negative for the presence of R-alleles, whereas frequencies between 50 and 88 % were determined for four fields in different geographical regions in the UK (Kent, Cambridgeshire, Oxfordshire and Wiltshire). The average R-allele frequency in UK populations sampled during spring 2003 (n = 65) was 28 %. In populations sampled from 13 fields in Northamptonshire an average R-allele frequency of 36 % was determined. Additionally, for 29 fields, R-allele frequencies were measured before and after treatment of fungicides (Table 10). For these fields, the average R-allele frequency increased from 27 to 92 % after fungicide treatments.

R-allele frequencies up to 100 % were measured for populations sampled throughout the UK during spring 2004 (Table 11 & Figure 27). Populations sampled in England (n = 22) showed R-allele frequencies between 67 and 100 %, with an average value of 88 %. The occurrence of high R-allele frequencies with less variation between regions can be explained by survival and further spread of resistant isolates from the previous season through production of ascospores. Lowest R-allele frequency, 49 %, was measured in a population sampled at Boghall Farm near Edinburgh. This field was subsequently used for one of the septoria leaf blotch core field experiments (see sections 4.5.1 and 4.5.2). In populations in Northern Ireland (n = 18), R-allele frequencies varied more than in England, between 6 and 100 %, with an average frequency of 60 %.

Figure 26. R-allele frequencies in *Septoria tritici* populations sampled in Ireland and the UK in spring 2003.

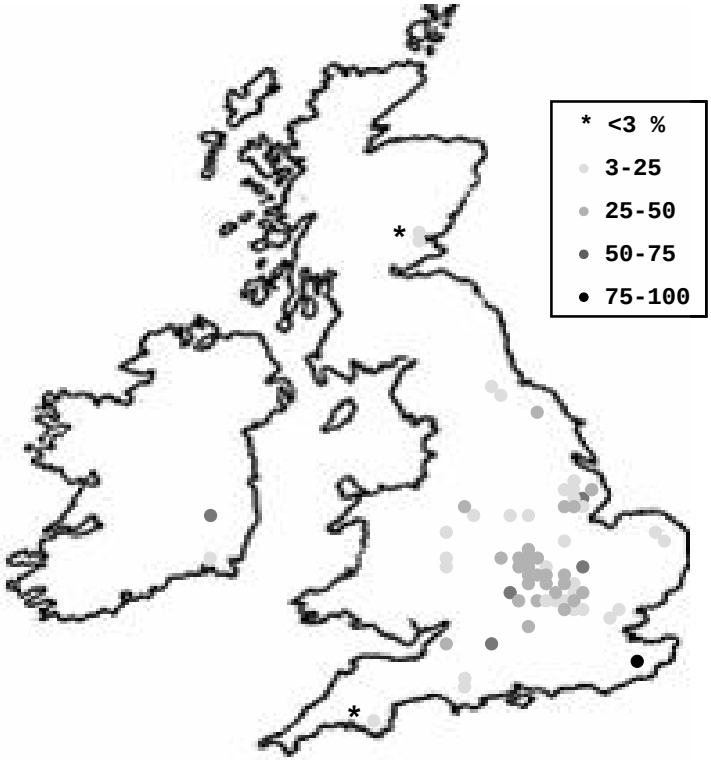
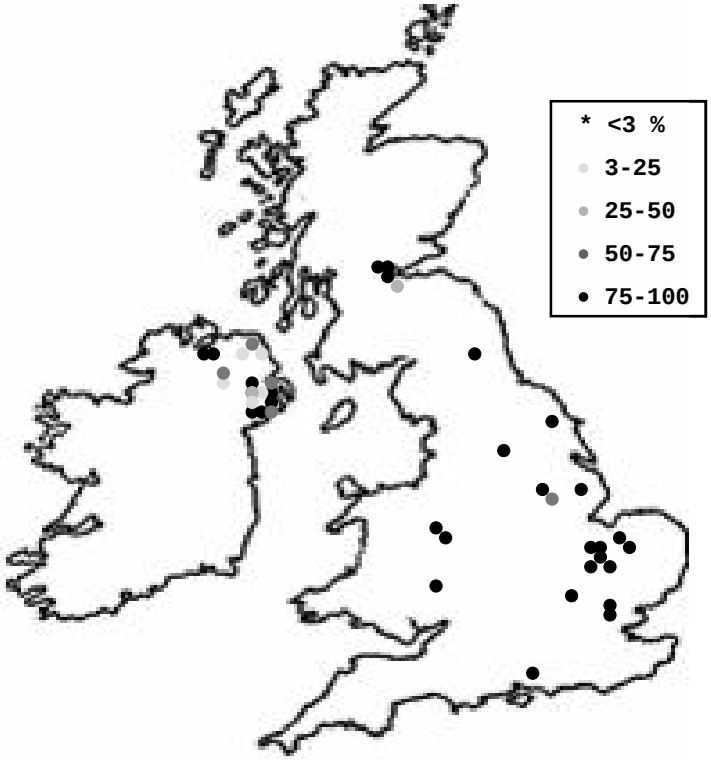


Table 11. Frequencies of R-alleles in UK populations of *S. tritici* sampled during spring 2004

Sample number	Location	Cultivar	Real-time PCR R-allele frequency
1	Rothamsted Research, Harpenden, Beds	Hereward	77
2	Boghall Farm, Kimming Hill, Edinburgh, Scotland	Consort	49
3	Bankhead Farm, Edinburgh, Scotland	Consort	81
4	Bankhead Farm, Edinburgh, Scotland	Consort	78
5	Bankhead Farm, Edinburgh, Scotland	Robigus	77
6	ADAS High Mowthorpe, Duggleby, Malton, N-Yorks	Consort	96
7	ADAS Boxworth, Cambridge, Cambs	Consort	100
8	ADAS Rosemaund, Preston Wynne, Hereford	Consort	78
9	ADAS Terrington, Kings Lynn, Norfolk	Consort	82
10	Steeple Mordon, Cambs	Consort	83
11	Steeple Mordon, Cambs	Consort	96
12	Shrewsbury, Shropshire	Tanker	91
13	Thaxted, Essex	Claire	83
14	Thaxted, Essex	Consort	93
15	Oakington, Cambs	Claire	86
16	Oakington, Cambs	Claire	77
17	Newport, Shropshire	Richmond	94
18	Hexham, Northumberland	Option	88
19	Brant Broughton, Lincolnshire	?	67
20	Horncastle, Lincolnshire	Claire	95
21	Downham Market, Norfolk	Tanker	91
22	Retford, Shefford	Claire	88
23	NIAB Headley Hall, Tadcaster, N-Yorks	?	76

24	Sutton Scotley, Winchester, Hampshire	?	100
25	South Willingham, Lincolnshire	?	94
26	NIAB Callow, Herefordshire	?	97
27	Co. Down, N-Ireland	Consort	79
28	Co. Down, N-Ireland	Consort	91
29	Co. Down, N-Ireland	Einstein	75
30	Co. Down, N-Ireland	Consort	82
31	Co. Londonderry, N-Ireland	Claire	100
32	Co. Down, N-Ireland	Claire	70
33	Co. Armagh, N-Ireland	Claire	71
34	Co. Down, N-Ireland	Consort	63
35	Co. Londonderry, N-Ireland	Exsept?	93
36	Co. Armagh, N-Ireland	Consort	6
37	Co. Antrim, N-Ireland	Claire	62
38	Co. Antrim, N-Ireland	Claire	24
39	Co. Antrim, N-Ireland	Savannah	20
40	Co. Down, N-Ireland	Napier	41
42	Co. Down, N-Ireland	Napier	6
43	Co. Down, N-Ireland	Claire	80
44	Co. Down, N-Ireland	Tanker	100
45	Co. Down, N-Ireland	Napier	21

Figure 27. R-allele frequencies in *S. tritici* populations sampled in the UK in spring 2004.



2.4.2 QoI resistance testing: correlation between populations and isolates

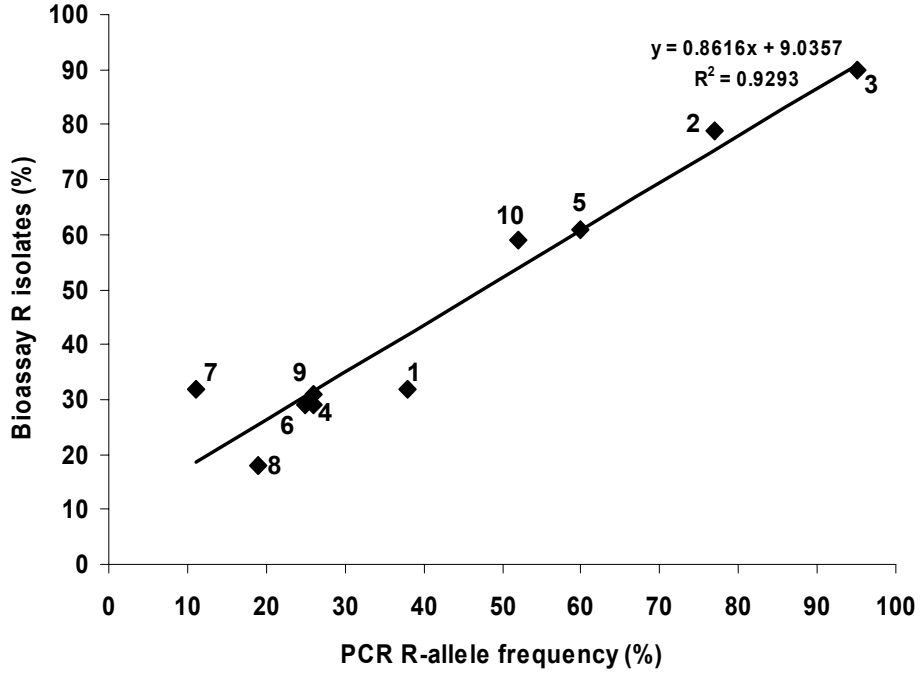
In ten samples, R-allele frequencies were simultaneously determined for populations by PCR, and individual isolates tested for fungicide sensitivity, respectively (Table 12). As expected, there was a strong linear relationship (R= 0.92) between the results of the two tests (Figure 28).

Table 12. Overview of samples for which R-allele frequencies were determined using PCR tests on populations and fungicide testing on isolates.

Sample number	Location/year	Cultivar	Real-time PCR R-allele frequency (%)	Proportion resistant isolates R/(S+R)
1	Rothamsted, Hertfordshire (2003)	Savannah	38	29/90
2	Rothamsted, Hertfordshire (2004)	Hereward	77	46/58
3	Rothamsted, Hertfordshire (2005)	Consort	95	43/48
4	Long Ashton, Somerset (2003)	Claire	26	15/52
5	Netherhampton, Wiltshire (2003)	?	60	14/23
6	Nuneaton, Warkwickshire (2003)	Claire	25	15/52
7	Newby, Middlesborough (2003)	Consort	11	16/50
8	Barton Farm, Sherborne, Dorset (2003)	Option	19	5/28
9	Chichley, Newport Bagnell, Buckinghamshire (2003)	Consort	26	10/32
10	Carlow, Ireland (2003)	Madrigal	52	19/32
11	Rothamsted, Hertfordshire (2006)	Robigus	nd	37/39

nd, not determined

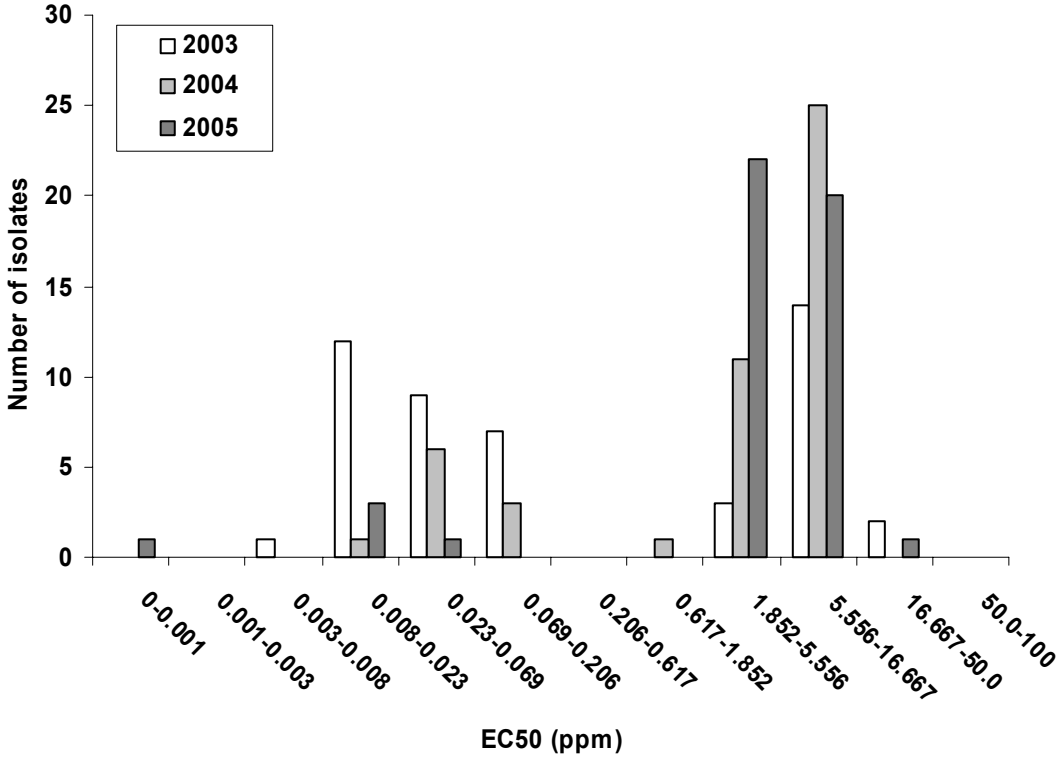
Figure 28. Relationship between R-allele frequencies determined in populations by PCR and fungicide sensitivity testing. The ten populations tested are listed in Table 12.



2.4.3 Distribution of azoxystrobin and epoxiconazole sensitivities within populations

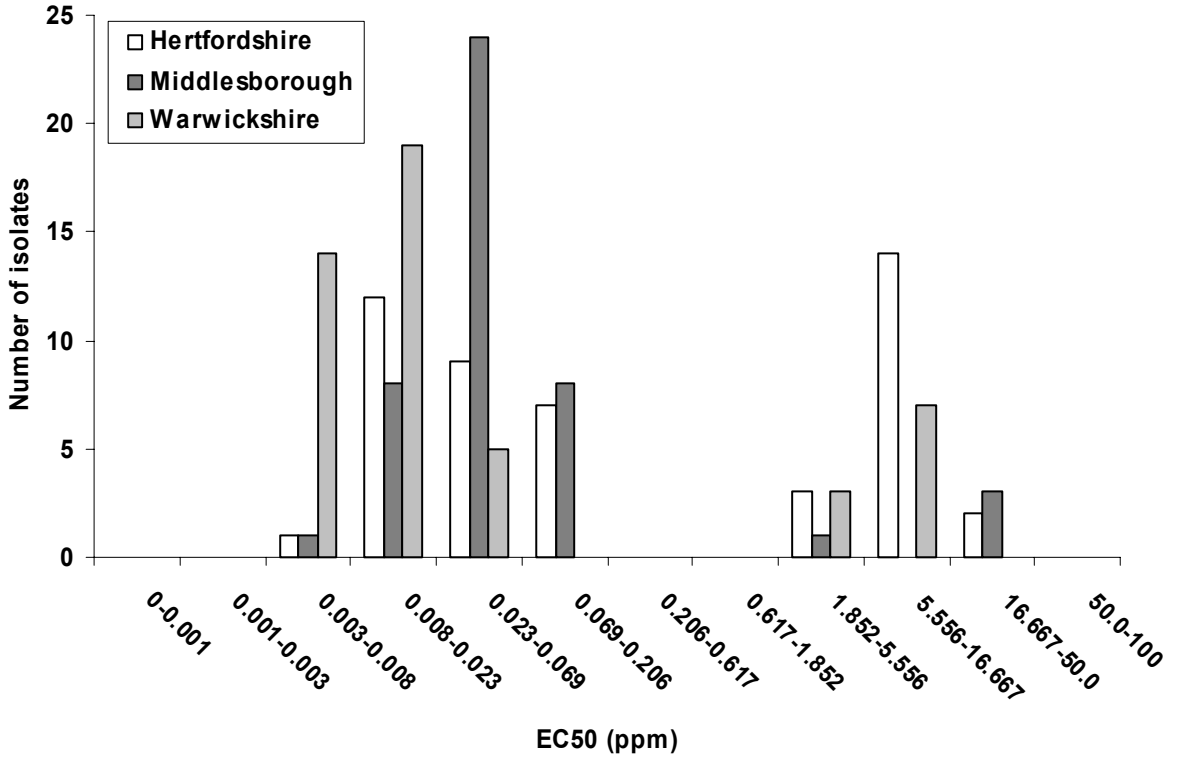
In three populations sampled at Rothamsted at the start of the growing season in 2003, 2004 and 2005, azoxystrobin sensitivities (EC_{50}) were determined for individual isolates (Figure 29A). The EC_{50} results showed two distinct groups, one with EC_{50} values between 0 to 0.206, and the other 0.617 to 50. Only R-alleles (A143) were detected in the isolates with higher EC_{50} values, whereas only S-alleles (G143) were found in isolates with lower EC_{50} values. As shown in Table 12, an increase in R-allele frequency was observed from 2003 onwards.

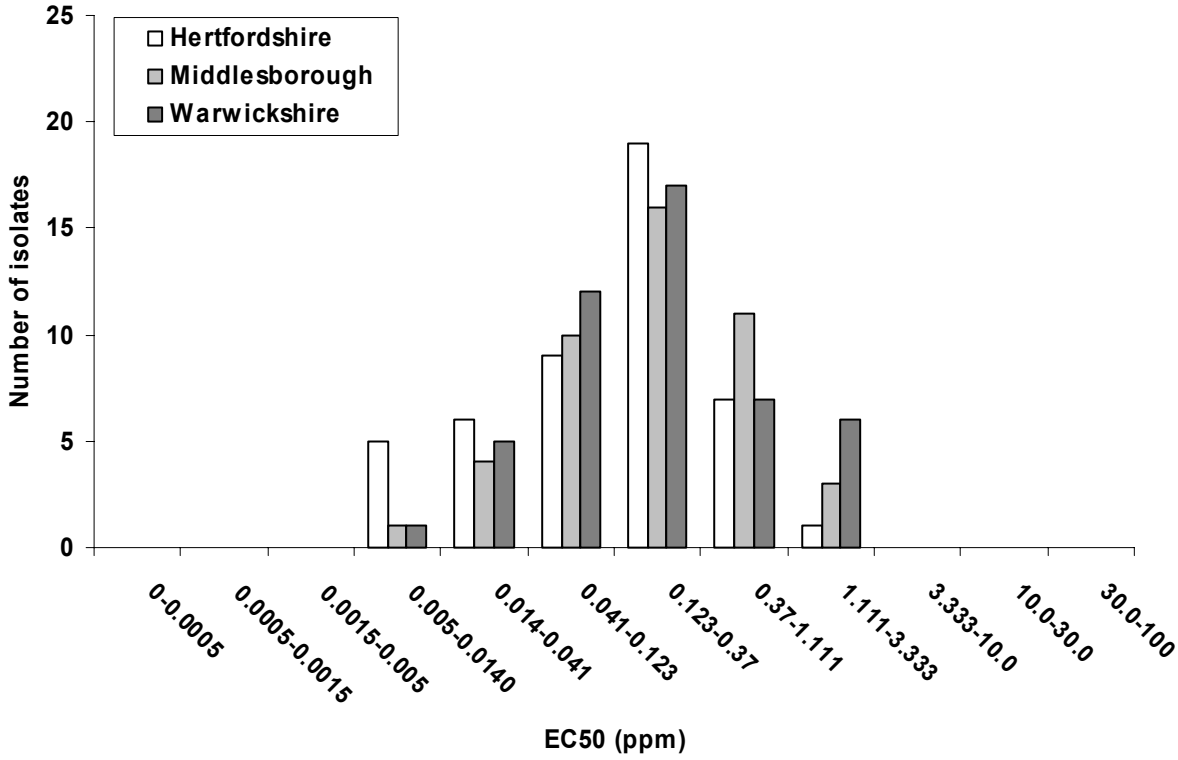
Figure 29A. Azoxystrobin EC_{50} profiles for populations sampled at Rothamsted during 2003-2005. In total, 48, 47 and 48 isolates were tested in 2003, 2004 and 2005 respectively.



Figures 30 A and B show the azoxystrobin and epoxiconazole EC_{50} profiles of populations sampled at Rothamsted (Hertfordshire), Newby (Middlesborough) and Nuneaton (Warwickshire) in 2003. A similar pattern was observed for all three locations. With regard to azoxystrobin sensitivity, there were two distinct subpopulations distinguished by the presence of either G143 or A143 cytochrome *b* alleles. For epoxiconazole, a wide range of sensitivities were measured, from 0.005 to 3.333 ppm. This pattern can be explained by the presence of multiple resistance mechanisms each contributing discretely to a less sensitive phenotype. The population sampled in Hertfordshire had a higher proportion of isolates in the more sensitive EC_{50} range, by comparison with the populations sampled in Middlesborough and Warwickshire.

Figure 30. Azoxystrobin (A) and epoxiconazole (B) EC_{50} profiles of populations sampled at Rothamsted (Hertfordshire, n = 48), Newby (Middlesborough, n = 45) and Nuneaton (Warwickshire, n = 48) in 2003.





2.5 Field trials conducted by agrochem industry partners

A series of field trials were carried out by industry partners. Syngenta investigated if QoI resistance development could be slowed down using different anti-resistance strategies based on fungicide mixtures, alternations and different spray timings. Results of trials carried out at three locations are presented in Tables 13A, B and C. Most disease developed at Waterford in Ireland (Table 13C). The initial R-allele frequency of 7 % measured before spraying was lower at this location than the others. At Waterford, the fungicide mixture programmes gave better disease control than repeated applications of single products. As solo product, Opus was far more effective than Bravo and Amistar. The mixture with Amistar and Bravo was less effective than Opus/Bravo, Opus/Amistar and Opus/Bravo/Amistar mixtures. With regard to QoI resistance development, all programmes with T1 and T2 applications of Amistar selected for populations with high levels of R-alleles. The highest level of R-alleles (73-90%) was measured for single product Amistar applications at T1 and T2; mixtures slowed down resistance development with the Bravo, Opus and Amistar mixture being most effective. Because of low disease levels measured in most samples obtained from both Lincolnshire sites, no conclusions about trends in level of disease control could be drawn. As there were only low levels of pathogen DNA in these samples (<100 pg per sample), R-allele frequencies measured might be inaccurate due to the limited number of lesions sampled, and hence the possibly small number of genotypes present. Where no visible lesions were present, samples with small amounts of pathogen DNA might represent a mixed population due to arrival of spores with many different genotypes.

Table 13A. Field trial results from location Metheringham in Lincolnshire (cv Claire).

Leaf layer	Amount of pathogen DNA (pg)	R-allele frequency (%)	T1 spray[1]	T2 spray	T3 spray
Flag	37	46	Untreated	Untreated	Untreated
L2	240	37	Untreated	Untreated	Untreated
L3	28	49	Untreated	Untreated	Untreated
Flag	5.8	79	Amistar (0.8)	Amistar (0.8)	Opus (0.4)
L2	3.3	47	Amistar (0.8)	Amistar (0.8)	Opus (0.4)
L3	nd	nd	Amistar (0.8)	Amistar (0.8)	Opus (0.4)
Flag	2.7	92	Opus (0.6)	Opus (0.6)	Opus (0.4)
L2	4.6	49	Opus (0.6)	Opus (0.6)	Opus (0.4)
L3	8	19	Opus (0.6)	Opus (0.6)	Opus (0.4)
Flag	<0.1	nd	Amistar (0.8) + Opus (0.6)	Amistar (0.8) + Opus (0.6)	Opus (0.4)
L2	6.2	79	Amistar (0.8) + Opus (0.6)	Amistar (0.8) + Opus (0.6)	Opus (0.4)
L3	65	65	Amistar (0.8) + Opus (0.6)	Amistar (0.8) + Opus (0.6)	Opus (0.4)
Flag	0.3	30	Amistar (0.8) + Opus (0.6) + Bravo (1.0)	Amistar (0.8) + Opus (0.6) + Bravo (1.0)	Opus (0.4)
L2	3.6	nd	Amistar (0.8) + Opus (0.6) + Bravo (1.0)	Amistar (0.8) + Opus (0.6) + Bravo (1.0)	Opus (0.4)
L3	260	29	Amistar (0.8) + Opus (0.6) + Bravo (1.0)	Amistar (0.8) + Opus (0.6) + Bravo (1.0)	Opus (0.4)
Flag	1.3	37	Amistar (0.8) + Opus (0.6)	Opus (0.6) + Bravo (1.0)	Amistar (0.6) + Opus (0.4)
L2	9	nd	Amistar (0.8) + Opus (0.6)	Opus (0.6) + Bravo (1.0)	Amistar (0.6) + Opus (0.4)
L3	11	nd	Amistar (0.8) + Opus (0.6)	Opus (0.6) + Bravo (1.0)	Amistar (0.6) + Opus (0.4)
Flag	<0.1	21	Opus (0.6) + Bravo (1.0)	Amistar (0.8) + Opus (0.6)	Amistar (0.6) + Opus (0.4)
L2	0.2	nd	Opus (0.6) + Bravo (1.0)	Amistar (0.8) + Opus (0.6)	Amistar (0.6) + Opus (0.4)
L3	76	12	Opus (0.6) + Bravo (1.0)	Amistar (0.8) + Opus (0.6)	Amistar (0.6) + Opus (0.4)

[1] Between brackets the rate of each fungicide in l/ha.

Table 13B. Field trial results from location Awards in Lincolnshire (cv Tanker).

Leaf layer	Amount of pathogen DNA (pg)	R-allele frequency (%)	T1 spray [1]	T2 spray	T3 spray
Flag	6.6	85	Untreated	Untreated	Untreated
L2	2.3	65	Untreated	Untreated	Untreated
L3	3	31	Untreated	Untreated	Untreated
Flag	0.2	77	Amistar (0.8)	Amistar (0.8)	Opus (0.4)
L2	nd	nd	Amistar (0.8)	Amistar (0.8)	Opus (0.4)
L3	54	100	Amistar (0.8)	Amistar (0.8)	Opus (0.4)
Flag	1.8	11	Opus (0.6)	Opus (0.6)	Opus (0.4)
L2	2.4	92	Opus (0.6)	Opus (0.6)	Opus (0.4)
L3	68	28	Opus (0.6)	Opus (0.6)	Opus (0.4)
Flag	nd	nd	Amistar (0.8) + Opus (0.6)	Amistar (0.8) + Opus (0.6)	Opus (0.4)
L2	1.2	20	Amistar (0.8) + Opus (0.6)	Amistar (0.8) + Opus (0.6)	Opus (0.4)
L3	68	52	Amistar (0.8) + Opus (0.6)	Amistar (0.8) + Opus (0.6)	Opus (0.4)
Flag	nd	nd	Amistar (0.8) + Opus (0.6) + Bravo (1.0)	Amistar (0.8) + Opus (0.6) + Bravo (1.0)	Opus (0.4)
L2	nd	nd	Amistar (0.8) + Opus (0.6) + Bravo (1.0)	Amistar (0.8) + Opus (0.6) + Bravo (1.0)	Opus (0.4)
L3	280	30	Amistar (0.8) + Opus (0.6) + Bravo (1.0)	Amistar (0.8) + Opus (0.6) + Bravo (1.0)	Opus (0.4)
Flag	0.5	12	Amistar (0.8) + Opus (0.6)	Opus (0.6) + Bravo (1.0)	Amistar (0.6) + Opus (0.4)
L2	48	89	Amistar (0.8) + Opus (0.6)	Opus (0.6) + Bravo (1.0)	Amistar (0.6) + Opus (0.4)
L3	10	100	Amistar (0.8) + Opus (0.6)	Opus (0.6) + Bravo (1.0)	Amistar (0.6) + Opus (0.4)
Flag	14	8	Opus (0.6) + Bravo (1.0)	Amistar (0.8) + Opus (0.6)	Amistar (0.6) + Opus (0.4)
L2	11	21	Opus (0.6) + Bravo (1.0)	Amistar (0.8) + Opus (0.6)	Amistar (0.6) + Opus (0.4)
L3	9.4	60	Opus (0.6) + Bravo (1.0)	Amistar (0.8) + Opus (0.6)	Amistar (0.6) + Opus (0.4)

[1] Between brackets the rate of each fungicide in l/ha.

Table 13C. Field trial results from location Waterford in Ireland (cv Claire).

Leaf layer	Amount of pathogen DNA (pg)	R-allele frequency (%)	T1 spray[1]	T2 spray	T3 spray
Flag	24000	7	Untreated	Untreated	Opus (0.6)
L2	7000	7	Untreated	Untreated	Opus (0.6)
L3	370	7	Untreated	Untreated	Opus (0.6)
Flag	1100	93	Amistar (0.8)	Amistar (0.8)	Opus (0.6)
L2	15000	73	Amistar (0.8)	Amistar (0.8)	Opus (0.6)
L3	19000	75	Amistar (0.8)	Amistar (0.8)	Opus (0.6)
Flag	620	34	Opus (0.6)	Opus (0.6)	Opus (0.6)
L2	4900	9	Opus (0.6)	Opus (0.6)	Opus (0.6)
L3	4500	12	Opus (0.6)	Opus (0.6	Opus (0.6)
Flag	1300	14	Bravo (1.5)	Bravo (1.5)	Opus (0.6)
L2	22000	9	Bravo (1.5)	Bravo (1.5)	Opus (0.6)
L3	9000	11	Bravo (1.5)	Bravo (1.5)	Opus (0.6)
Flag	11	27	Amistar (0.8) + Opus (0.6)	Amistar (0.8) + Opus (0.6)	Opus (0.6)
L2	650	53	Amistar (0.8) + Opus (0.6)	Amistar (0.8) + Opus (0.6)	Opus (0.6)
L3	2500	62	Amistar (0.8) + Opus (0.6)	Amistar (0.8) + Opus (0.6)	Opus (0.6)
Flag	680	88	Amistar (0.8) + Bravo (1.5)	Amistar (0.8) + Bravo (1.5)	Opus (0.6)
L2	7000	53	Amistar (0.8) + Bravo (1.5)	Amistar (0.8) + Bravo (1.5)	Opus (0.6)
L3	14000	52	Amistar (0.8) + Bravo (1.5)	Amistar (0.8) + Bravo (1.5)	Opus (0.6)
Flag	4	76	Amistar (0.8) + Opus (0.6) + Bravo (1.5)	Amistar (0.8) + Opus (0.6) + Bravo (1.5)	Opus (0.6)
L2	130	39	Amistar (0.8) + Opus (0.6) + Bravo (1.5)	Amistar (0.8) + Opus (0.6) + Bravo (1.5)	Opus (0.6)
L3	3100	12	Amistar (0.8) + Opus (0.6) + Bravo (1.5)	Amistar (0.8) + Opus (0.6) + Bravo (1.5)	Opus (0.6)
Flag	20	10	Opus (0.6)+ Bravo (1.5)	Amistar (0.8) + Opus (0.6)	Opus (0.6)
L2	980	35	Opus (0.6)+ Bravo (1.5)	Amistar (0.8) + Opus (0.6)	Opus (0.6)
L3	8100	14	Opus (0.6)+ Bravo (1.5)	Amistar (0.8) + Opus (0.6)	Opus (0.6)
Flag	18	33	Amistar (0.8) + Opus (0.6)	Opus (0.6)+ Bravo (1.5)	Opus (0.6)
L2	130	40	Amistar (0.8) + Opus (0.6)	Opus (0.6)+ Bravo (1.5)	Opus (0.6)
L3	4500	41	Amistar (0.8) + Opus (0.6)	Opus (0.6)+ Bravo (1.5)	Opus (0.6)
Flag	7	3	Opus (0.6)+ Bravo (1.5)	Opus (0.6)+ Bravo (1.5)	Amistar (0.8) + Opus (0.6)
L2	56	15	Opus (0.6)+ Bravo (1.5)	Opus (0.6)+ Bravo (1.5)	Amistar (0.8) + Opus (0.6)
L3	1300	11	Opus (0.6)+ Bravo (1.5)	Opus (0.6)+ Bravo (1.5)	Amistar (0.8) + Opus (0.6)

[1] Between brackets the rate of each fungicide in l/ha.

Field trials conducted by BASF showed high levels of QoI resistance in Oxfordshire, with R-frequencies of 45 and 52 %, and low and/or undetectable levels of QoI resistance in Scotland and Devon, with R-allele frequencies between <3 and 22 %, at the start of the season in 2003 (Table 14). Fungicide treatments resulted in high levels of disease control and increased levels of green leaf area throughout the season. For all sites, a significant increase in R-allele frequency was measured after application of fungicides, with values up to 90 % for most locations, whereas the R-allele frequency remained low for populations sampled from the untreated plots.

Table 14. Effect of fungicide sprays on QoI resistance development in *Septoria tritici* populations sampled from different areas geographical areas within the UK.

Site[1]	Leaf layer	Growth stage	Treated[2]	Amount of pathogen DNA (pg)	R-allele frequency (%)	Visual symptoms (%)	Green Leaf Area (%)
Abernethy	All	32	-	270	<3	0.9	nd
Fallaw	All	32	-	890	22	1.7	nd
Chillington	All	32	-	9800	<3	11.8	nd
Yealmpton	All	32	-	5600	10	5.1	nd
Stratton Audley	All	32	-	1000	45	5.6	nd
Weston on the Green	All	32	-	410	52	3.4	nd
Abernethy	Flag	39	+	0.5	17	0.0	100
Abernethy	L2	39	+	4	84	0.3	99.7
Abernethy	L3	39	+	86	43	1.5	98.5
Abernethy	L4	39	+	930	64	9.4	90.6
Fallaw	Flag	39	+	<0.1	nd	0.0	100
Fallaw	L2	39	+	<0.1	nd	0.2	98.8
Fallaw	L3	39	+	9.9	46	1.0	98.7
Fallaw	L4	39	+	1400	42	11.6	88.4
Chillington	Flag	39	+	1.7	19	0.0	100
Chillington	L2	39	+	14	26	0.1	99.9
Chillington	L3	39	+	4.7	25	0.2	99.8
Chillington	L4	39	+	nd	29	6.1	90.5
Yealmpton	Flag	39	+	0.1	23	0.0	100
Yealmpton	L2	39	+	<0.1	nd	0.0	100
Yealmpton	L3	39	+	12	83	0.3	99.7
Yealmpton	L4	39	+	nd	54	4.1	94.0
Stratton Audley	Flag	39	+	6.5	46	0.0	99.1
Stratton Audley	L2	39	+	6.4	56	0.3	98.9
Stratton Audley	L3	39	+	430	80	2.3	95.7
Stratton Audley	L4	39	+	1800	74	17.0	72.0
Weston on the Green	Flag	39	+	18	95	0.0	100
Weston on the Green	L2	39	+	79	99	0.1	99.2
Weston on the Green	L3	39	+	300	93	0.2	99.5
Weston on the Green	L4	39	+	710	97	2.4	82.7
Abernethy	Flag	59	+	0.03	52	0.2	99.8
Abernethy	L2	59	+	nd	74	0.6	95
Abernethy	L3	59	+	31	22	2.1	94.4
Abernethy	L4	59	+	2100	66	11.1	63.5
Fallaw	Flag	59	+	<0.1	nd	0.1	99.9
Fallaw	L2	59	+	<0.1	nd	1.8	98.2
Fallaw	L3	59	+	8.8	88	3.8	96.2
Fallaw	L4	59	+	310	84	31.0	52.5
Chillington	Flag	59	+	1.1	30	0.2	99.8
Chillington	L2	59	+	8.2	56	1.2	98.4
Chillington	L3	59	+	nd	41	5.1	93.1
Chillington	L4	59	+	18	49	44.2	50.5
Yealmpton	Flag	59	+	0.2	46	0.0	100
Yealmpton	L2	59	+	1.8	82	0.6	99.2
Yealmpton	L3	59	+	26	88	2.3	96.7
Yealmpton	L4	59	+	240	72	6.7	85.1
Stratton Audley	Flag	59	+	nd	72	1.3	87.8
Stratton Audley	L2	59	+	270	87	3.9	80.5

Stratton Audley	L3	59	+	3800	82	37.5	0.75
Stratton Audley	L4	59	+	nd	nd	47.5	0
Weston on the Green	Flag	59	+	14	87	0.0	98.5
Weston on the Green	L2	59	+	76	87	1.1	96.7
Weston on the Green	L3	59	+	1300	96	3.9	94.0
Weston on the Green	L4	59	+	1600	96	16.3	66.5
Abernethy	Flag	75	+	nd	nd	5.0	95.0
Abernethy	L2	75	+	29	89	39.0	61.0
Abernethy	L3	75	+	1600	85	75.50	25.5
Abernethy	L4	75	+	nd	nd	95.3	4.7
Abernethy	Flag	75	-	11000	3	48.0	52.0
Abernethy	L2	75	-	9300	9	94.6	5.4
Abernethy	L3	75	-	nd	nd	95.5	4.5
Abernethy	L4	75	-	nd	nd	100	0
Fallaw	Flag	75	+	2.5	45	49.5	50.5
Fallaw	L2	75	+	nd	nd	75.5	24.5
Fallaw	L3	75	+	nd	91	98.3	1.7
Fallaw	L4	75	+	nd	nd	100	0
Fallaw	Flag	75	-	16	79	96.0	4.0
Fallaw	L2	75	-	1	47	96.5	3.5
Fallaw	L3	75	-	nd	nd	100	0
Fallaw	L4	75	-	nd	nd	100	0
Chillington	Flag	75	+	3.6	24	3.6	92.4
Chillington	L2	75	+	5000	30	45.7	48.5
Chillington	L3	75	+	430	38	94	4.2
Chillington	L4	75	+	nd	nd	100	0
Chillington	Flag	75	-	nd	nd	100	0
Chillington	L2	75	-	13000	3	100	0
Chillington	L3	75	-	nd	nd	100	0
Chillington	L4	75	-	nd	nd	100	0
Yealmpton	Flag	75	+	620	82	0.8	98.3
Yealmpton	L2	75	+	nd	nd	10.2	85.5
Yealmpton	L3	75	+	2300	79	43.5	50.0
Yealmpton	L4	75	+	nd	nd	90.3	7.7
Yealmpton	Flag	75	-	26000	8	100	0
Yealmpton	L2	75	-	44000	18	100	0
Yealmpton	L3	75	-	nd	nd	100	0
Yealmpton	L4	75	-	nd	nd	100	0

nd; not determined.

[1] Sites Abernethy (cv Riband) and Fallaw (cv Claire) are in Scotland; Chillington (cv. Equinox) and Yealmpton (cv. Reapers) in Devon; Stratton Audley (cv Consort) and Weston on the Green (cv Option) in Oxfordshire.

[2] Treatment consisted of two sprays of Opera (a.i. pyraclostrobin and epoxiconazole) and one spray of Caramba (a.i. metconazole).

DuPont conducted field trials at three different sites in 2003 (Tables 15, 16 and 17). The main aim was to investigate if different fungicide programmes can influence QoI resistance development in field populations of *Septoria tritici*. The level of Septoria leaf blotch during the growing season was much lower in Warwickshire than in Ireland and Kent. The highest R-allele frequencies in untreated plots for populations sampled from different leaf layers at GS 39 were measured in Kent, with frequencies between 82 and 89 %, followed by Ireland and Warwickshire with frequencies of 54 to 67 and 17 to 35 %, respectively. Generally, highest level of disease control was achieved with the mixture of flusilazole, pyraclostrobin and

chlorothalonil. Mixtures of flusilazole and pyraclostrobin and coded fungicide and pyraclostrobin were also effective. The R-allele frequency increased significantly in all treated plots with the exception of the flusilazole treated plots where like the untreated plots only a small increase in R-allele frequency was measured at the end of the season.

Table 15. Effect of fungicide treatments on QoI-resistance development in *Septoria tritici* populations sampled in Kent. A, PCR quantification of *Septoria tritici* in samples; B, R-allele frequencies determined for leaf populations using G143A as marker.

A									
Treatments[1]	**Amount of pathogen DNA (pg)**								
	GS 39	**GS 39**	**GS 39**	**GS 59**	**GS 59**	**GS 59**	**GS 75**	**GS 75**	**GS 75**
	L2	**L3**	**L4**	**L1**	**L2**	**L3**	**L1**	**L2**	**L3**
1	9.6	5.2	40	86	2700	31000	6400	54000	nd
2	37	38	160	480	2600	18000	3700	65000	nd
3	8	6.4	79	710	510	35000	7500	390000	nd
4	1.3	28	15	3.7	540	35000	22000	110000	nd
5	14	37	150	5.3	490	10000	nd	66000	nd
6	12	12	57	270	4600	36000	5800	76000	nd
7	2.4	24	140	nd	780	13000	7300	53000	nd
8	nd	nd	nd	16	1100	60000	13000	120000	nd

B									
Treatments[1]	**R-allele frequency (%)**								
	GS 39	**GS 39**	**GS 39**	**GS 59**	**GS 59**	**GS 59**	**GS 75**	**GS 75**	**GS 75**
	L2	**L3**	**L4**	**L1**	**L2**	**L3**	**L1**	**L2**	**L3**
1	82	89	86	93	94	100	100	100	nd
2	97	92	93	88	92	94	98	99	nd
3	96	92	94	95	94	99	100	100	nd
4	95	95	72	94	96	100	100	100	nd
5	98	97	98	97	100	100	98	100	nd
6	91	95	92	92	97	95	100	100	nd
7	98	97	97	nd	95	99	100	100	nd
8	nd	nd	nd	89	94	100	100	100	nd

nd, not determined

[1] Treatment 1, untreated; 2, two sprays of flusilazole; 3, two sprays of pyraclostrobin; 4, two sprays of mixture with flusilazole and pyraclostrobin; 5, two sprays of mixture with chlorothalonil, flusilazole and pyraclostrobin; 6, two sprays of coded fungicide; 7, two sprays of mixture with coded fungicide and pyraclostrobin; 8, one spray of mixture with coded fungicide and pyraclostrobin. Sprays applied at T1 and T2.

Table 16. Effect of fungicide treatments on QoI-resistance development in *Septoria tritici* populations sampled in Ireland. A, PCR quantification of *Septoria tritici* in samples; B, R-allele frequencies determined for leaf populations using G143A as marker.

A									
Treatments[1]	**Amount of pathogen DNA (pg)**								
	GS 39	**GS 39**	**GS 39**	**GS 59**	**GS 59**	**GS 59**	**GS 75**	**GS 75**	**GS 75**
	L2	**L3**	**L4**	**L1**	**L2**	**L3**	**L1**	**L2**	**L3**
1	90	13000	47000	21000	32000	60000	12000	63000	nd
2	69	13000	17000	2400	14000	32000	16000	nd	nd
3	910	nd	8100	7100	13000	34000	33000	nd	39000
4	590	16000	9200	3200	3400	44000	13000	70000	28000
5	570	nd	3000	4200	4200	33000	nd	nd	nd
6	660	nd	8600	nd	8500	39000	29000	nd	nd
7	440	5200	11000	8400	28000	53000	37000	nd	nd
8	2300	nd	37000	2800	33000	nd	38000	nd	nd

B									
Treatments[1]	**R-allele frequency (%)**								
		GS39			**GS59**			**GS75**	
	L2	**L3**	**L4**	**L1**	**L2**	**L3**	**L1**	**L2**	**L3**
1	67	54	63	53	73	67	75	73	nd
2	62	56	56	68	71	65	67	nd	nd
3	98	77	91	86	73	83	100	nd	100
4	100	96	93	95	97	100	99	100	100
5	97	nd	90	97	92	99	nd	nd	nd
6	75	59	46	76	88	82	96	100	nd
7	93	95	90	100	100	100	100	100	nd
8	64	55	51	76	82	nd	97	nd	nd

nd, not determined

[1] Treatment 1, untreated; 2, two sprays of flusilazole; 3, two sprays of pyraclostrobin; 4, two sprays of mixture with flusilazole and pyraclostrobin; 5, two sprays of mixture with chlorothalonil, flusilazole and pyraclostrobin; 6, two sprays of coded fungicide; 7, two sprays of mixture with coded fungicide and pyraclostrobin; 8, one spray of mixture with coded fungicide and pyraclostrobin. Sprays applied at T1 and T2.

Table 17. Effect of fungicide treatments on QoI-resistance development in *Septoria tritici* populations sampled in Warwickshire. A, PCR quantification of *Septoria tritici* in samples; B, R-allele frequencies determined for leaf populations using G143A as marker.

A									
Treatments[1]	**Amount of pathogen DNA (pg)**								
		GS39			**GS59**			**GS75**	
	L2	**L3**	**L4**	**L1**	**L2**	**L3**	**L1**	**L2**	**L3**
1	1.2	23	0.3	9	29	nd	80	5200	nd
2	<0.1	1.9	38	2.2	nd	nd	36	430	39000
3	nd	1.6	nd	10	9.7	380	430	9400	nd
4	1.8	6.3	35	25	nd	500	nd	1100	18000
5	<0.1	6.7	nd	35	4.6	55	11	130	1700
6	<0.1	1	55	0.5	0.2	370	24	5100	nd
7	<0.1	3.1	nd	0.6	1.2	240	87	55	nd
8	<0.1	51	nd	49	13	270	11	760	36000

B									
Treatments[1]	**R-allele frequency (%)**								
		GS39			**GS59**			**GS75**	
	L2	**L3**	**L4**	**L1**	**L2**	**L3**	**L1**	**L2**	**L3**
1	35	17	34	42	46	50	41	64	nd
2	nd	33	64	30	58	33	45	55	76
3	nd	23	64	nd	79	96	100	100	100
4	70	64	69	18	79	96	nd	100	nd
5	nd	61	59	9	33	88	47	100	83
6	nd	60	49	nd	nd	82	75	83	nd
7	nd	31	58	nd	61	100	75	100	nd
8	nd	20	26	nd	15	100	67	93	100

nd, not determined

[1] Treatment 1, untreated; 2, two sprays of flusilazole; 3, two sprays of pyraclostrobin; 4, two sprays of mixture with flusilazole and pyraclostrobin; 5, two sprays of mixture with chlorothalonil, flusilazole and pyraclostrobin; 6, two sprays of coded fungicide; 7, two sprays of mixture with coded fungicide and pyraclostrobin; 8, one spray of mixture with coded fungicide and pyraclostrobin. Sprays applied at T1 and T2.

Bayer investigated the effect of different spray timings of strobilurin and azole fungicides on QoI resistance development (Table 18A, B, C and D). All leaf samples tested represented large *Septoria tritici* populations with the exception of the early season sample send from Lincolnshire in which only 68 pg of pathogen DNA was detected in 50 ng of total DNA extracted from leaves. In comparison with the site in Five, Scotland, where an R-allele frequency of 81 % was measured at the start of the season, the R-allele frequencies for populations sampled from two sites in Norfolk were much lower with values of 48 and 29 %. Because of the rapid development of QoI resistance, with R-allele frequencies between 85 and 100 % measured for all population sampled from strobilurin-treated plots at all sites, no differential effects of spray timing of azoles and strobilurins on QoI resistance development were observed. As expected, the R-allele frequency measured in untreated and azole-only treated plots remained generally lower.

In another trial conducted at Morley, the effect of wheat cultivar on QoI-resistance development was studied. The differences between R-allele frequencies measured for populations sampled from the ten different cultivars were too small to see a clear cultivar effect. The highest R-allele frequency, 49 %, was measured for the population sampled from cv Option, whereas the lowest R-allele frequency, 18 %, was measured for the population sampled from cv. Solstice. A high cultivar resistance rating was not linked with a low R-allele frequency.

Table 18. Effect of spray timing of fungicides on QoI resistance development in *Septoria tritici* populations.

A.Location Heathersett, Norfolk

Sample	Growth Stage	QoI spray timing	Triazole spray timing	Amount of pathogen DNA (pg)	R-allele frequency (%)
Overall sample	GS 32	Untreated	Untreated	12000	48
L2	GS 75	Untreated	Untreated	18000	39
L2	GS 75	Untreated	T1 T2 T3	1900	37
L2	GS 75	T2	T1 T2 T3	3000	100
L2	GS 75	T1 T2	T1 T2 T3	4600	100
L2	GS 75	T2 T3	T1 T2 T3	5600	100
L2	GS 75	T1 T3	T1 T2 T3	3300	85
L2	GS 75	T1 T2	Untreated	5800	94

B. Location Morley, Norfolk

Sample	Growth Stage	QoI spray timing	Triazole spray timing	Amount of pathogen DNA (pg)	R-allele frequency (%)
Overall sample	GS 32	Untreated	Untreated	4200	29
L2	GS 75	Untreated	Untreated	4800	75
L2	GS 75	Untreated	T1 T2 T3	510	57
L2	GS 75	T2	T1 T2 T3	1700	100
L2	GS 75	T1 T2	T1 T2 T3	1100	98
L2	GS 75	T2 T3	T1 T2 T3	1500	93
L2	GS 75	T1 T3	T1 T2 T3	660	100
L2	GS 75	T1 T2	Untreated	5500	100

C. Location Newton, Lincolnshire

Sample	Growth Stage	QoI spray timing	Triazole spray timing	Amount of pathogen DNA (pg)	R-allele frequency (%)
Overall sample	GS 32	Untreated	Untreated	68	53
L2	GS 75	Untreated	Untreated	8600	100
L2	GS 75	Untreated	T1 T2 T3	11000	98
L2	GS 75	T2	T1 T2 T3	11000	100
L2	GS 75	T1 T2	T1 T2 T3	8500	100
L2	GS 75	T2 T3	T1 T2 T3	16000	100
L2	GS 75	T1 T3	T1 T2 T3	7300	100
L2	GS 75	T1 T2	Untreated	56000	100

D. Location Fife, Scotland

Sample	Growth Stage	QoI spray timing	Triazole spray timing	Amount of pathogen DNA (pg)	R-allele frequency (%)
Overall sample	GS 32	Untreated	Untreated	14000	81
L2	GS 75	Untreated	Untreated	19000	100
L2	GS 75	Untreated	T1 T2 T3	9100	100
L2	GS 75	T2	T1 T2 T3	10000	100
L2	GS 75	T1 T2	T1 T2 T3	14000	100
L2	GS 75	T2 T3	T1 T2 T3	15000	100
L2	GS 75	T1 T3	T1 T2 T3	7700	100
L2	GS 75	T1 T2	Untreated	5300	100

Table 19. Effect of cultivars on QoI resistance development in populations of *Septoria tritici* sampled at Morley, Norfolk.

Wheat cultivar	Growth Stage	Fungicide treatment	Amount of pathogen DNA (pg)	R-allele frequency (%)
Access	GS 59-75	Untreated	18000	**40**
Claire	GS 59-75	Untreated	3200	**30**
Consort	GS 59-75	Untreated	9300	**34**
Deben	GS 59-75	Untreated	9400	**23**
Malacca	GS 59-75	Untreated	4700	**39**
Napier	GS 59-75	Untreated	5600	**38**
Option	GS 59-75	Untreated	13000	**49**
Robigus	GS 59-75	Untreated	2300	**19**
Solstice	GS 59-75	Untreated	8100	**18**
Tanker	GS 59-75	Untreated	3700	**43**

Although several field trials were carried out by the industry partners in 2004, only samples collected at the start of the season were tested for QoI resistance levels with PCR. Because of the high R-allele frequencies at the start of the season, with all populations sampled showed frequencies > 90%, further testing of samples for QoI resistance during the growing season was not carried out.

2.6 Septoria core field experiments in 2003 and 2004

2.6.1 Effect of dose and spray number on QoI-resistance development

Location Terrington in 2003

The average R-allele frequency in *Septoria tritici* populations sampled from whole plants before spraying was approximately 25 % at GS 32. Sampling at GS 39, after application of the first spray, revealed that R-allele frequencies had increased markedly in plots treated with Comet (Figure 31). Low frequencies were found in plots with a one-spray programme because these plots were as yet unsprayed(see Table 5). R-allele frequencies between 95 and 100 % were measured for populations sampled from all treated plots, independent of dose rate or number of Comet sprays. In comparison with the populations sampled in early spring, the average R-allele frequencies measured for populations sampled from untreated plots were double by the end of the season with values between 51 and 61 %. Highest R-allele frequencies in the untreated plots were measured for populations sampled from the flag leaves.

Disease assessments using PCR showed no clear effect of dose or number of Comet sprays on the level of disease control after the first two sprays (Figure 32). Assessment of the final disease levels at GS 75 showed a beneficial effect of multiple sprays when comparing identical fungicide inputs (1.0, 2.0 or 3.0 l/ha). Highest level of disease control was achieved with three sprays of 0.33 l/ha Comet followed by two sprays of 0.66 l/ha and a single spray of 1.0 l/ha. The advantage of using multiple sprays decreased with increasing fungicide inputs.

Visual assessment of disease in the upper two leaves at GS 75 showed that disease levels were very low (Figure 33). The disease levels recorded for flag leaves were negligible and only up to 5 % of the leaf area was covered with disease for some Leaf 2 samples. When comparing spray programmes with identical fungicide inputs (total dose of 1.0, 2.0 or 3.0 l/ha), highest levels of disease on Leaf 2 were measured for plots that had received a single spray of Comet

In comparison with the untreated plots no clear effect on Green Leaf Area and yield was observed for the different spray programmes (Figure 34). The levels of GLA were between 90 and 100 % for all samples whereas for all plots yields yield between 10.5 and 11.2 tonnes per hectare were measured. The highest average yield was recorded for the plots treated with three sprays of 1.0 l/ha of Comet.

Figure 31. Effect of fungicide applications on QoI resistance development in populations of *Septoria tritici*. R-allele frequencies for populations sampled from different leaf layers after the 1st (A), 2nd (B) and 3rd spray (C) at GS 39, 59 and 75, respectively. Spray number and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.

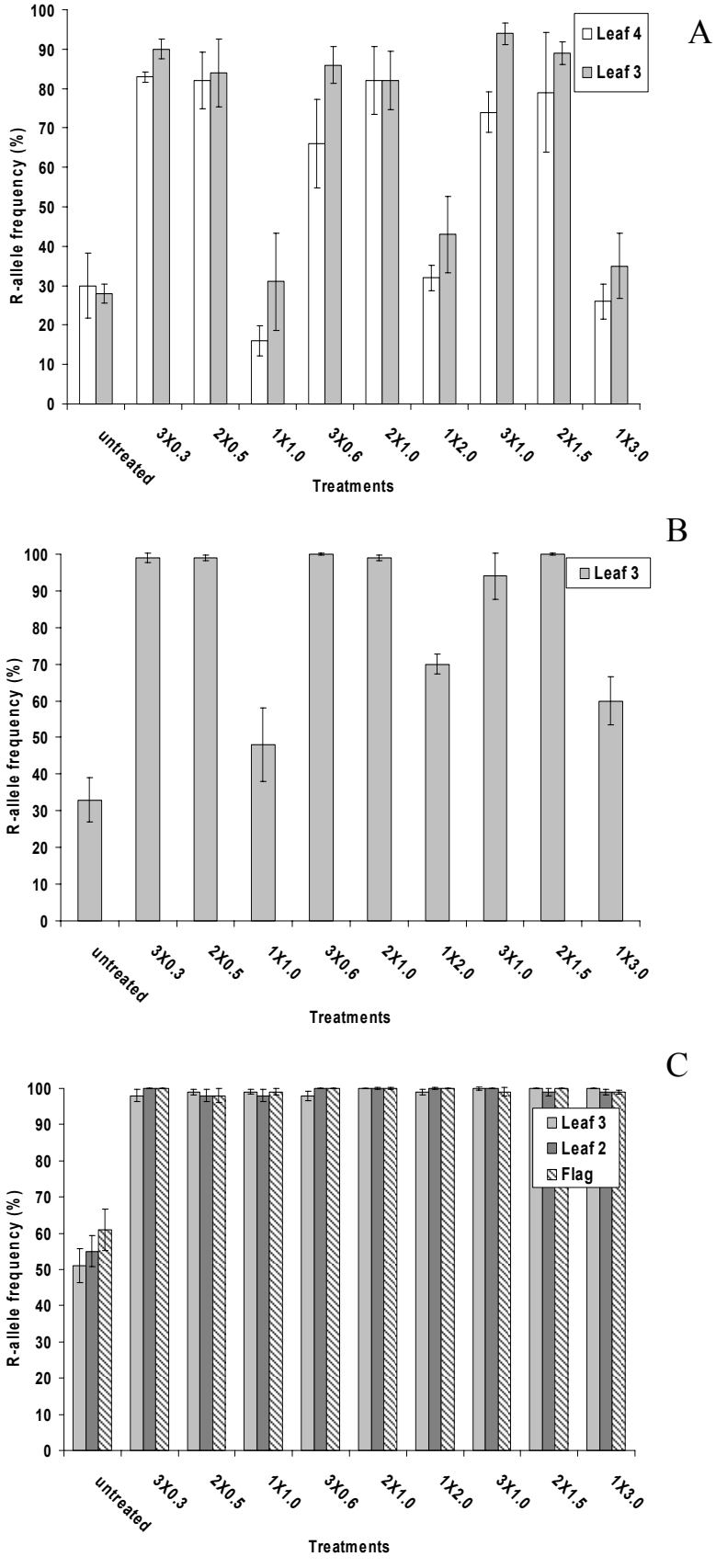


Figure 32. Effect of fungicide applications on Septoria infection levels as determined by PCR. Pathogen DNA levels in leaf samples after the 1st (A), 2nd (B) and 3rd spray (C) at GS 39, 59 and 75, respectively. Spray number and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.

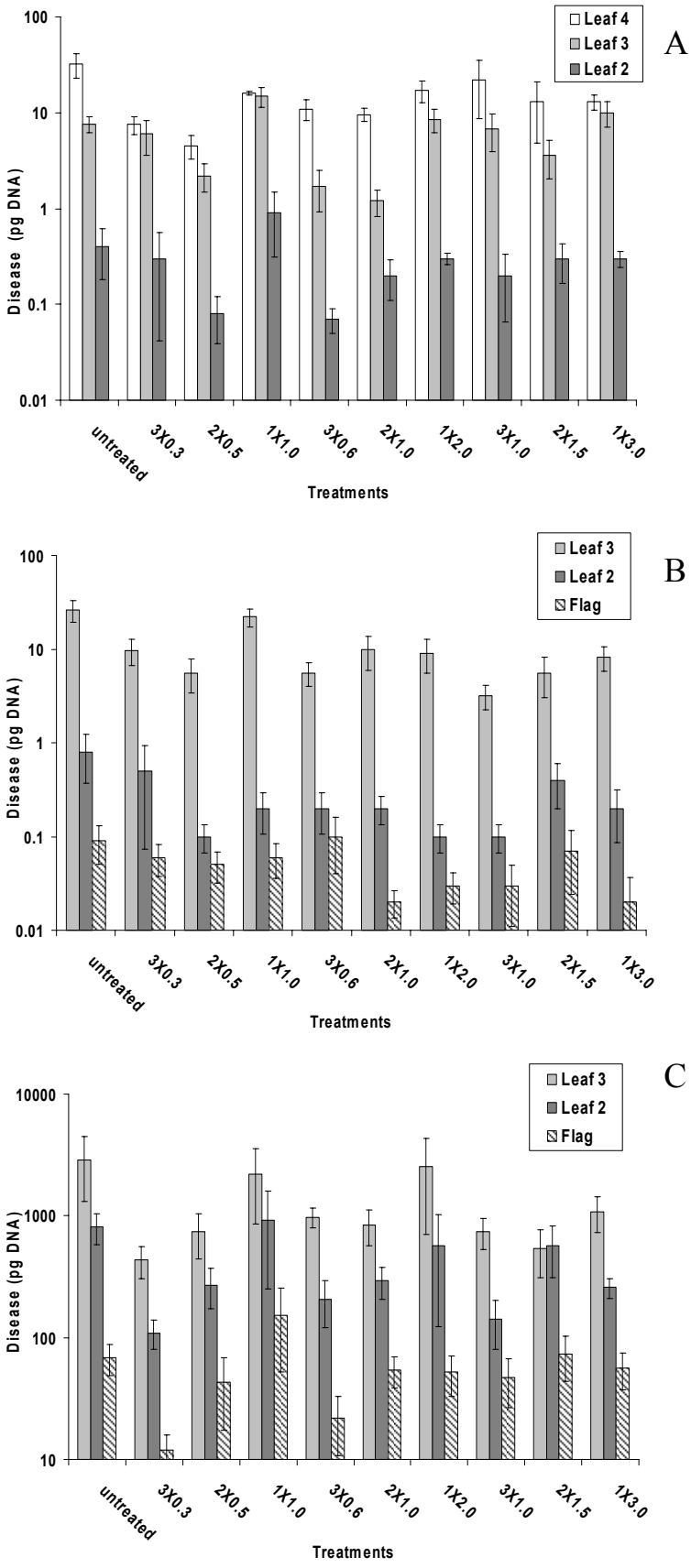


Figure 33. Effect of fungicide applications on Septoria disease levels as determined by visual assessments. Disease levels on Leaf 2 and flag at GS 75 are presented. Spray number and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.

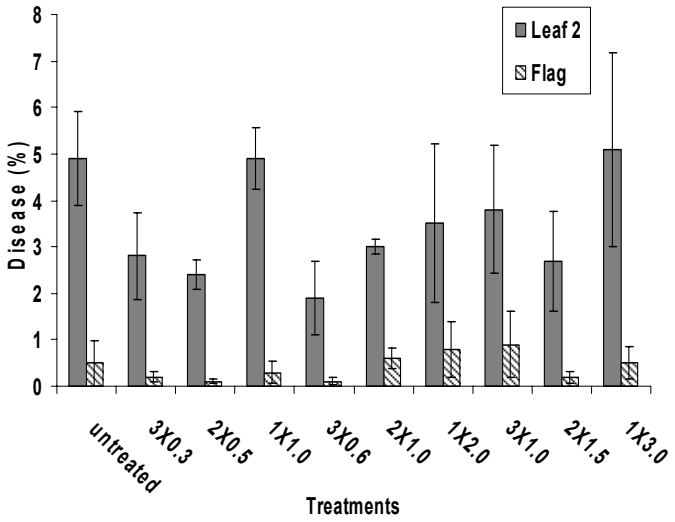
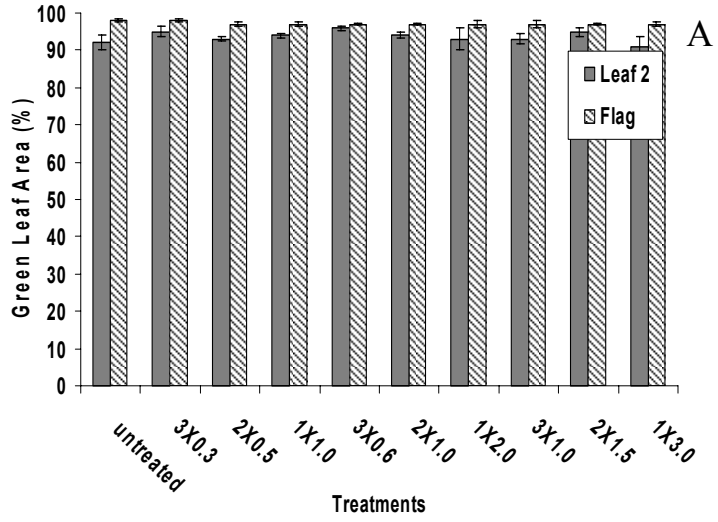
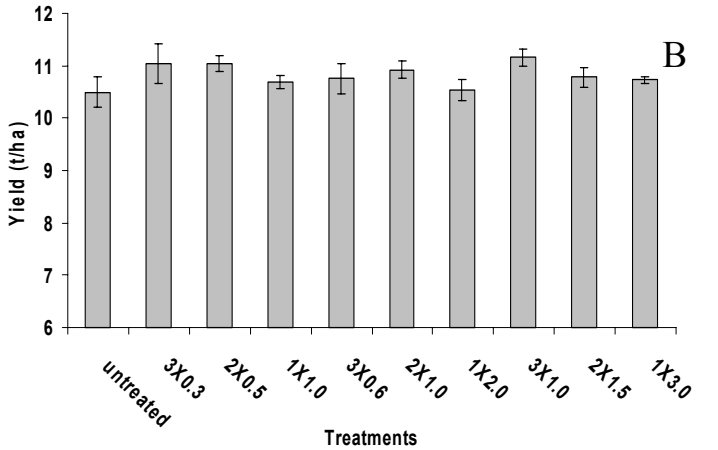


Figure 34. Effect of fungicide applications on Green Leaf Area and yield. GLA of Leaf 2 and flag at GS 75 (A) and yields (B). Spray number and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.





Location Dundee in 2003

R-allele frequencies between 3 and 20 % were measured in *Septoria tritici* populations (n = 37) sampled from whole plants before spraying at Dundee at GS 32. The average R-allele frequency was 9 %.

Sampling at GS 39, after application of the first spray, revealed that average R-allele frequencies were only increased significantly in plots treated with Comet (Figure 35). These frequencies were between 29 and 57 %, with the highest frequencies measured in plots sprayed with high rates of Comet (≥1.0 l/ha). In addition to the untreated plots, low frequencies between 7 and 32 % were also found in plots with a one-spray programme because these plots were as yet unsprayed (sprayed later at the same time as the second spray application) (see Table 5). R-allele frequencies measured after the second and third spray showed a clear effect of spray number. Highest frequencies were recorded for the three spray programmes, followed by the two and single spray programmes, respectively. In comparison with plots that had received a total dose of Comet of 2.0 and 3.0 l/ha, R-allele frequencies were lower in plots which had received a total dose of 1.0 l/ha.

Disease assessments using PCR showed, with the exception of three times of Comet at 0.66 l/ha, lower levels of disease in all treated plots after the first spray (Figure 36). After the second spray, the disease levels in the treated plots were much lower than those recorded for the untreated plots. Assessment of the final disease levels at GS 75 showed generally a beneficial effect of multiple sprays when comparing identical fungicide inputs (1.0, 2.0 or 3.0 l/ha) with regard to disease control, particularly for Leaf 2 and 3. There was no significant difference in disease control between the different doses of Comet applied.

Visual assessment of disease in the upper two leaves at GS 75 showed that the disease levels measured were very low, less than 3.5 %, with the exception of the untreated plots and plots that had only received a single spray of Comet of 1.0 l/ha (Figure 37). There were no significant differences between the remaining treated plots.

Figure 35. Effect of fungicide applications on QoI resistance development in *Septoria tritici* populations. R-allele frequencies for populations sampled from different leaf layers after the 1st (A), 2nd (B) and 3rd spray (C) at GS 39, 59 and 75, respectively. Number of sprays and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.

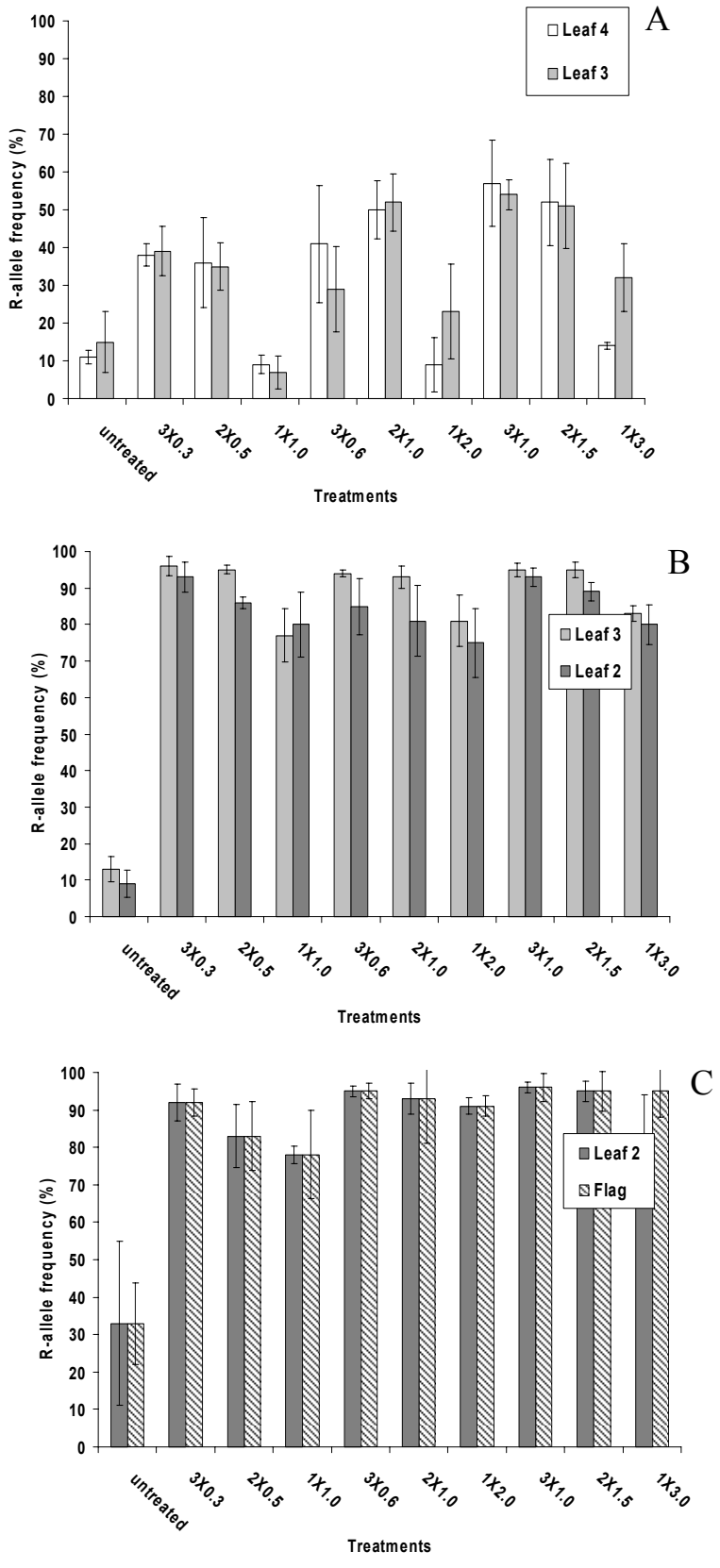


Figure 36. Effect of fungicide applications on Septoria infection levels as determined by PCR. Pathogen DNA levels in leaf samples after the 1st (A), 2nd (B) and 3rd spray (C) at GS 39, 59 and 75, respectively. Number of sprays and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.

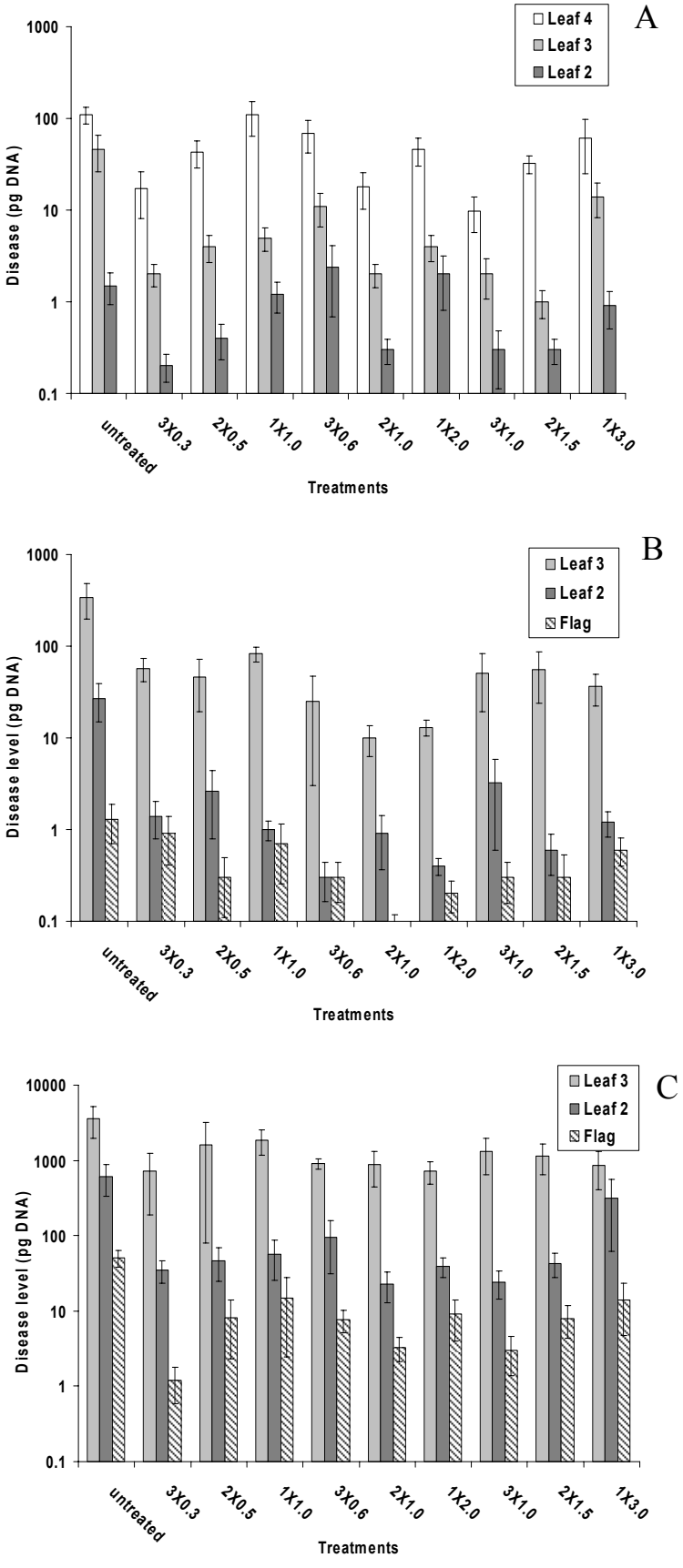
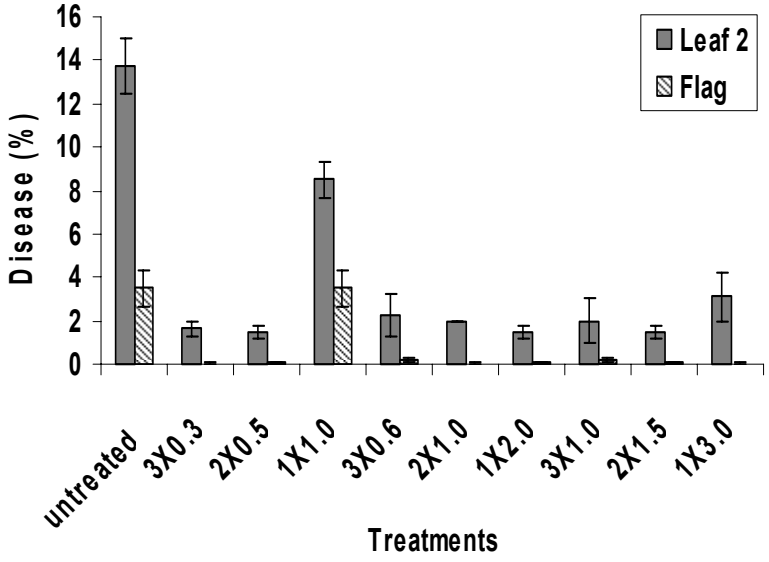
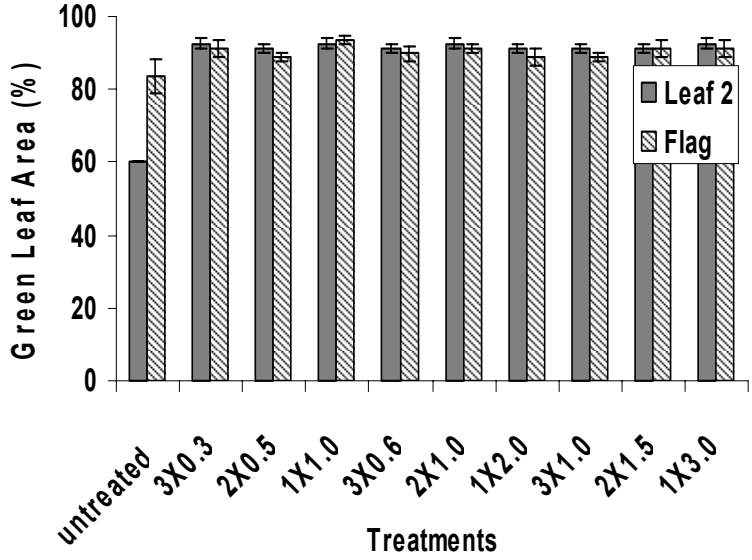


Figure 37. Effect of fungicide applications on Septoria disease levels as determined by visual assessments. Disease levels for Leaf 2 and flag at GS 75 are presented. Spray number and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.



Lowest average levels of GLA (Figure 38), 60 % for Leaf 2 and 84 % for the flag, were measured in the untreated plots due to disease and early senescence. There were no significant differences in GLA between the remaining treated plots with all values between 89 and 93 %.

Figure 38. Effect of fungicide applications on Green Leaf Area at GS 75. Spray number and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.



Location Terrington in 2004

R-allele frequencies between 69 and 82 % were measured in *Septoria tritici* leaf populations (n = 40) sampled from whole plants before spraying at GS 32. The average frequency of R-alleles in populations was approximately 76 %.

Sampling at GS 39, after application of the first spray, revealed that R-allele frequencies between 93 and 100 % were measured in all plots treated with Comet (Figure 39). Lower R-allele frequencies between 77 and 84 % were found in untreated plots and plots with a one-spray programme because these plots were sprayed later at the same time as the second spray application (see Table 5). After the second and third spray only R-allele frequencies between 94 and 100 % were measured for populations sampled from treated plots. A relatively high R-allele frequency of 92 % was measured for populations sampled from flag leaves of untreated plots at the end of the season.

Disease assessments using PCR after the first and second spray did not show showed lower disease levels in treated plots in comparison with the untreated plots (Figure 40). Only assessments of the final disease levels on flags at GS 75 showed a beneficial effect of multiple sprays when a total dose of Comet of 3.0 l/ha was applied. There was a slight positive total dose effect on disease control when comparing the two and three spray programmes.

Visual assessment of disease in the upper two leaves at GS 75 showed that disease levels were very high in comparison with the previous season (Figure 41). There was a positive dose effect; higher rates increased disease control but an increased spray number seemed to have a negative impact on disease control when the lower total doses of Comet of 1.0 and 2.0 l/ha were used. It is possible that high rates of Comet can partially control isolates carrying G143A alleles, but the level of control is inferior to the Opus based treatments carried out in the same field trial (see Figure 57).

In comparison with the untreated plots, all fungicide treatments increased the GLA of the two upper leaves (Figure 42A). The level of GLA was negatively correlated with disease level; there was a positive dose effect; higher rates of Comet increased GLA but an increased spray number had a negative impact on GLA at a low total Comet input of 1.0 or 2.0 l/ha. In comparison with the yield of the untreated plots (6.6 t/ha), the yield increases measured for the treated plots were between 1.0 and 1.7 t/ha (Figure 42B). The yields obtained with a total dose of 1.0 l/ha of Comet were significant lower than those obtained with 2.0 or 3.0 l/ha. The highest yield, 8.3 t/ha, was obtained with three sprays of Comet of 1.0 l/ha. However, higher levels of GLA and yields were obtained with treatments based on Opus applications carried out in the same location (see Figure 58).

Figure 39. Effect of fungicide applications on QoI resistance development in *Septoria tritici* populations. R-allele frequencies for populations sampled from different leaf layers after the 1st (A), 2nd (B) and 3rd spray (C) at GS 39, 59 and 75, respectively. Number of sprays and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.

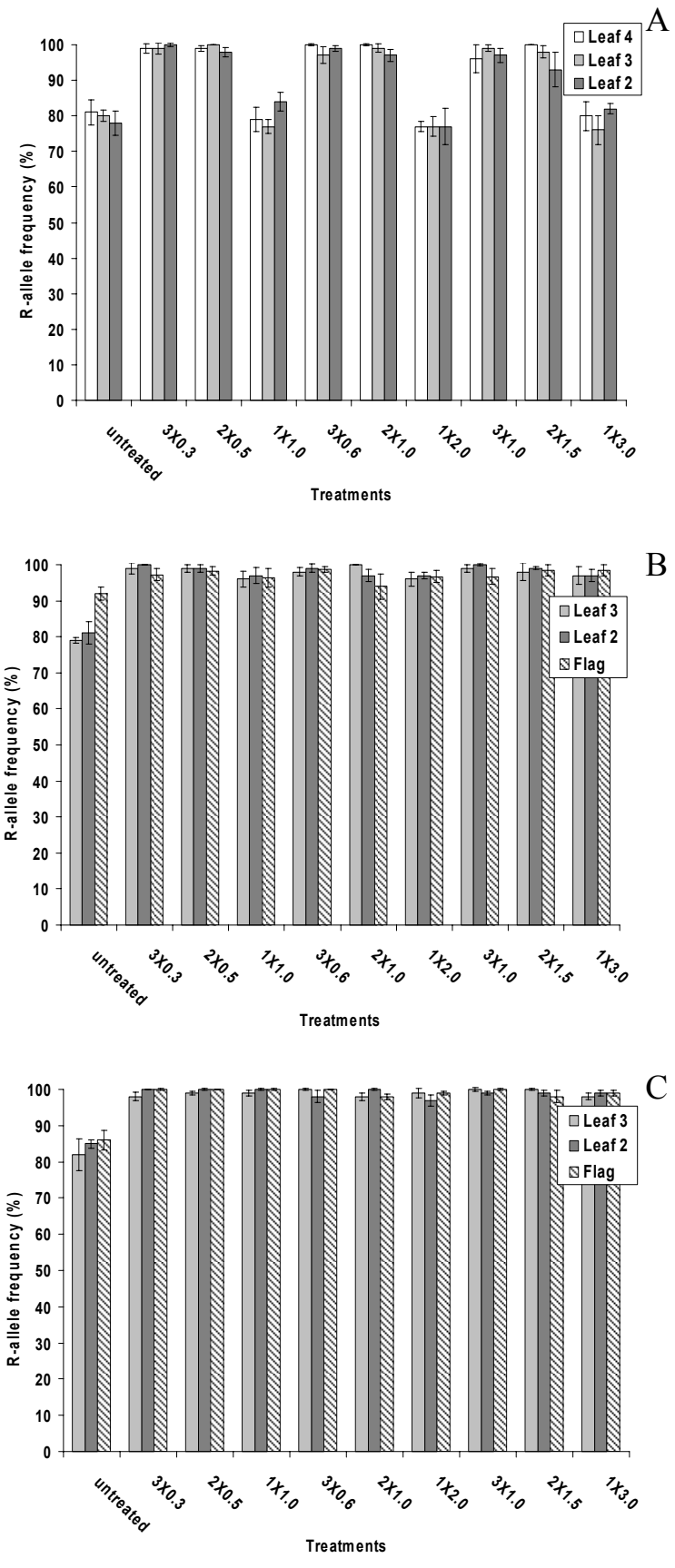


Figure 40. Effect of fungicide applications on Septoria infection levels as determined by PCR. Pathogen DNA levels in leaf samples after the 1st (A), 2nd (B) and 3rd spray (C) at GS 39, 59 and 75, respectively. Spray number and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.

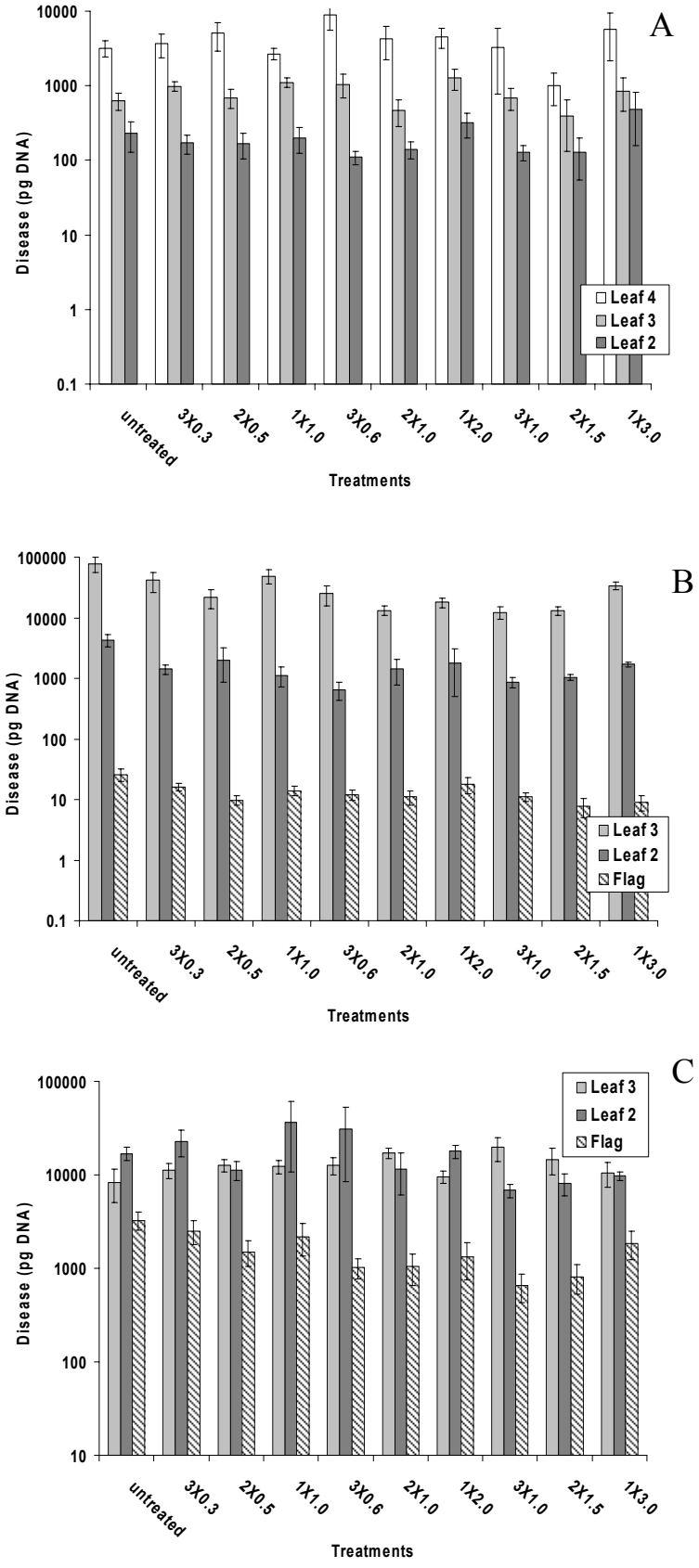


Figure 41. Effect of fungicide applications on Septoria disease levels as determined by visual assessments. Disease levels on Leaf 2 and flag at GS 75 are presented. Number of sprays and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.

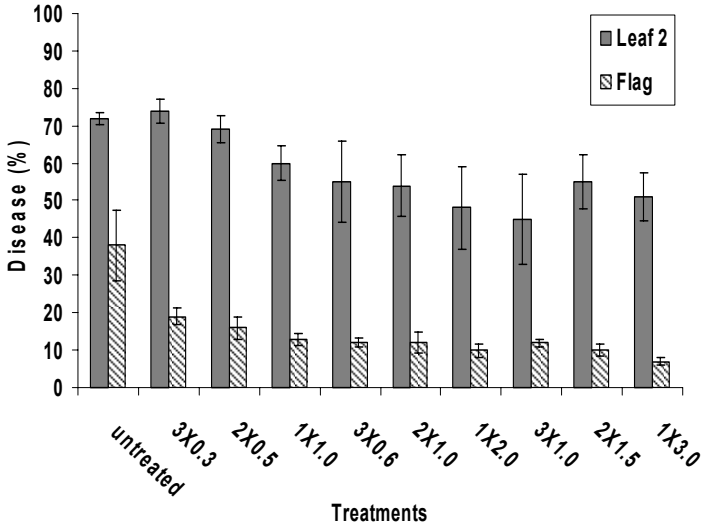
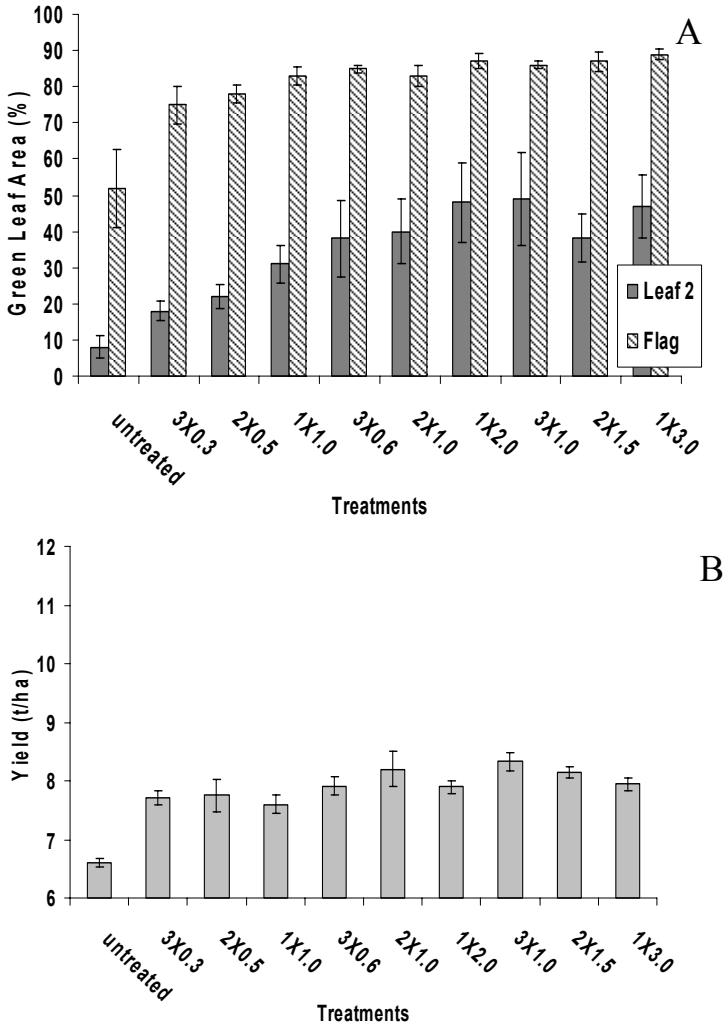


Figure 42. Effect of fungicide applications on Green Leaf Area and yield. GLA of Leaf 2 and flag at GS 75 (A) and yields (B). Number of sprays and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.



Location Edinburgh in 2004

R-allele frequencies between 17 and 35 % were measured in *Septoria tritici* populations (n = 40) sampled from whole plants before spraying at GS 32. The average R-allele frequency was approximately 26 %. Sampling at GS 39, after application of the first spray, revealed that R-allele frequencies increased in plots treated with Comet (Figure 43) with frequencies recorded between 85 and 95 %. Besides the untreated plots, low frequencies were also found in plots with a one-spray programme because these plots were as yet unsprayed (sprayed later at the same time as the second spray application) (see Table 5). After the first spray of the single spray programmes a positive effect on the selection of R-alleles was observed with increasing dose. The highest frequencies were recorded for the plots treated with Comet at 3.0 l/ha. After the third spray, high R-allele frequencies between 91 and 100 % were measured for populations sampled from all treated plots, independent of dose rate or number of Comet sprays. An average R-allele frequency of 34 % was measured for leaf populations sampled from the untreated plots at the end of the season.

Disease assessments using PCR showed no clear effect of dose or number of Comet sprays on the level of disease control after the first two sprays (Figure 44). Assessment of the final disease levels of flags at GS 75 showed a slight beneficial effect of total fungicide input with lowest levels of disease in plots treated with Comet at 3.0 l/ha.

Visual assessment of disease in the upper two leaves at GS 75 showed that disease levels were very low (Figure 45). The disease levels recorded for flag leaves were negligible and only up to 3 % of the leaf area was covered with disease for some Leaf 2 samples. In comparison with the untreated plots, all fungicide treatments resulted in lower levels of disease on Leaf 2. There was a slight positive effect of total dose on disease control. Disease was better controlled in plots sprayed with a total dose of 3.0 l/ha than in plots treated with 1.0 l/ha of Comet. Spray number had no effect on disease levels.

In comparison with the untreated plots, none of the treatments increased significantly the GLA (Figure 46A). For all plots, GLA levels of 73 to 83 % and 88 to 94 % were measured for Leaf 2 and flag samples, respectively. Most treatments, but not all, resulted in a yield increase (Figure 46B). The highest average yield of 6.9 t/ha was measured for plots that had received three sprays of Comet at 0.33 l/ha with the lowest average yields of 6.1 t/ha recorded for the untreated plots and plots that had received only a single spray of Comet at 1.0 l/ha.

Figure 43. Effect of fungicide applications on QoI resistance development in populations of *Septoria tritici*. R-allele frequencies in populations sampled from different leaf layers after the 1st (A), 2nd (B) and 3rd spray (C) at GS 39, 59 and 75, respectively. Spray number and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.

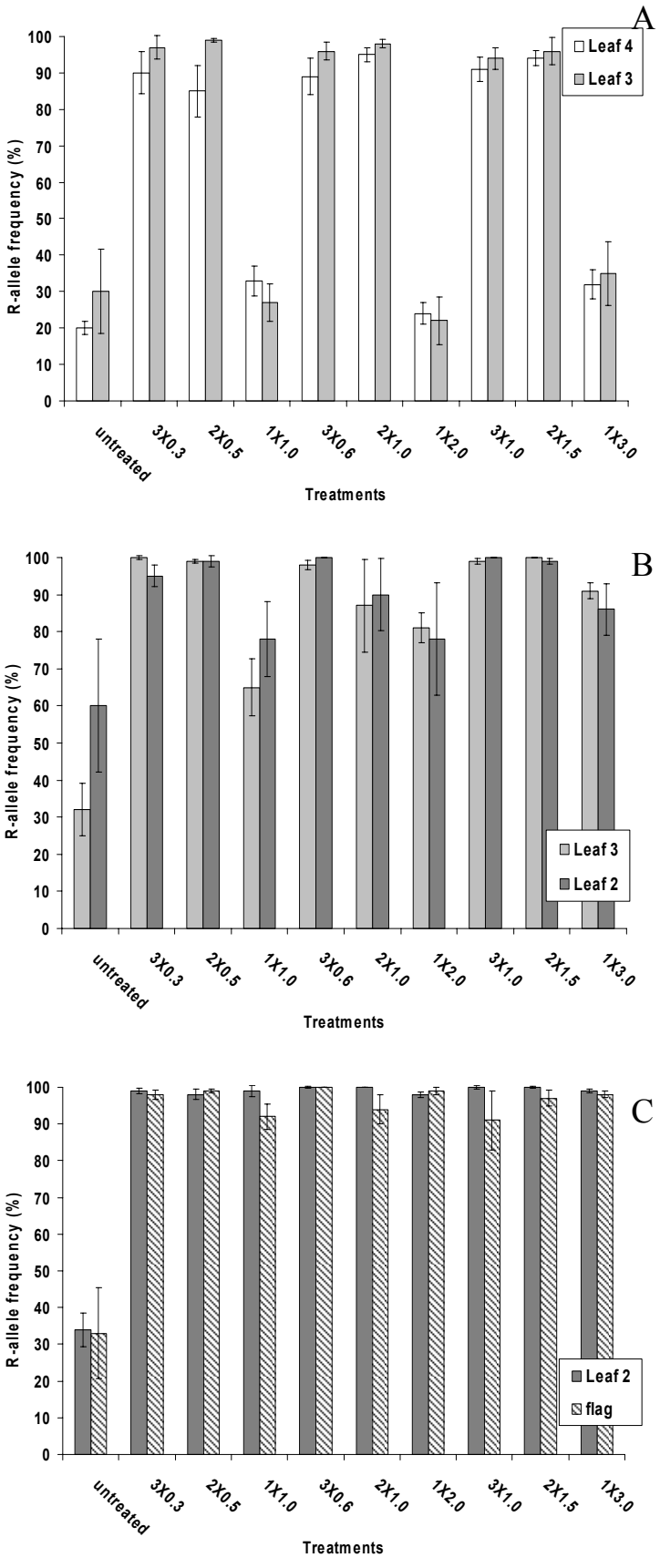


Figure 44. Effect of fungicide applications on Septoria infection levels as determined by PCR. Pathogen DNA levels in leaf samples after the 1st (A), 2nd (B) and 3rd spray (C) at GS 39, 59 and 75, respectively. Spray number and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.

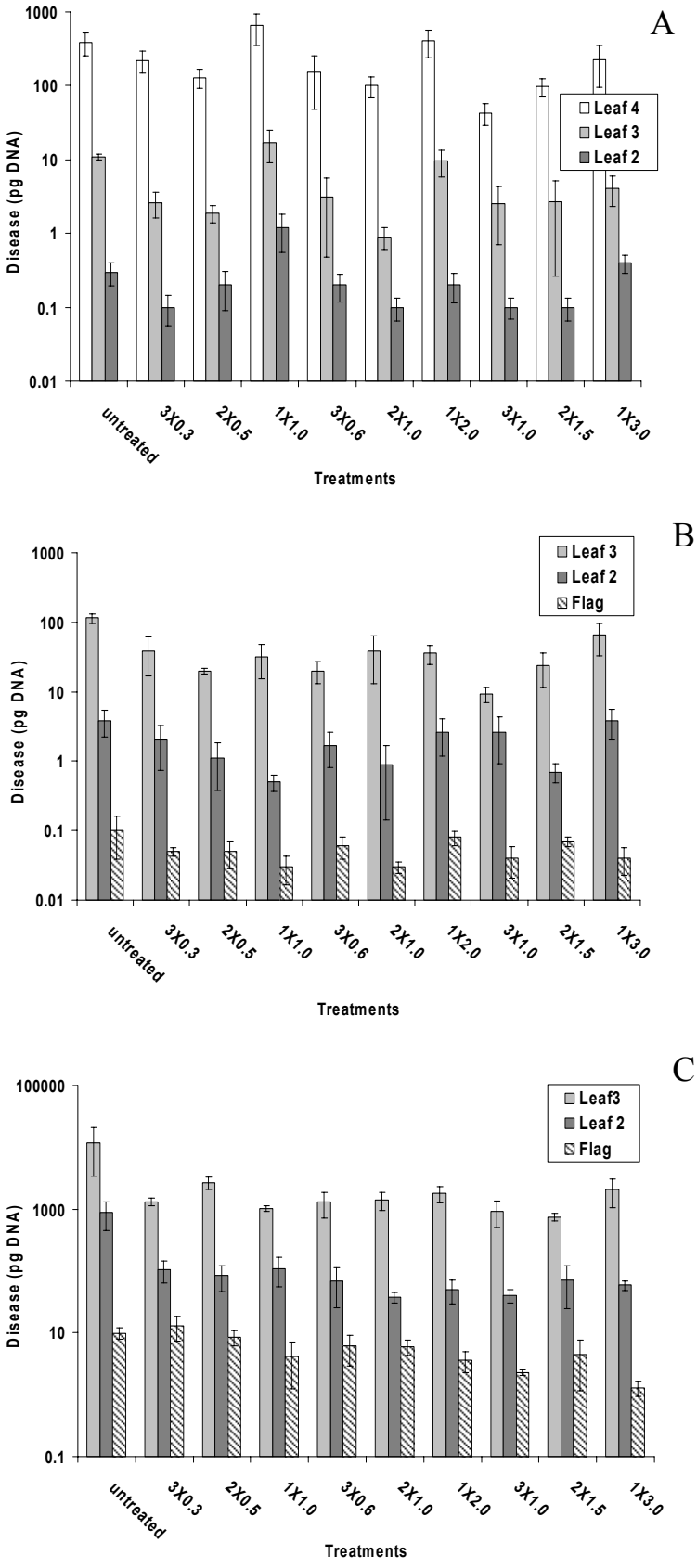


Figure 45. Effect of fungicide applications on Septoria disease levels as determined by visual assessments. Disease levels on Leaf 2 and flag at GS 75 are presented. Number of sprays and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.

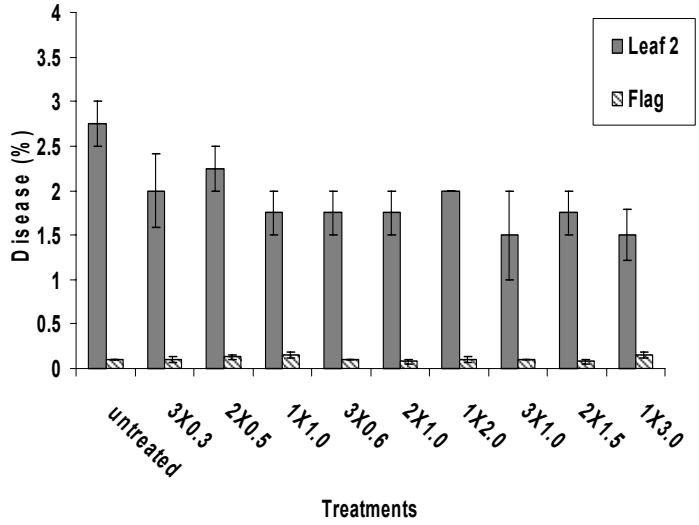
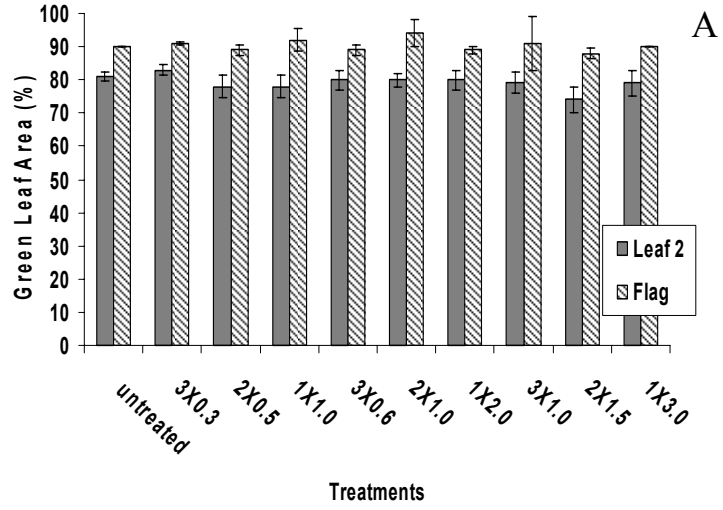
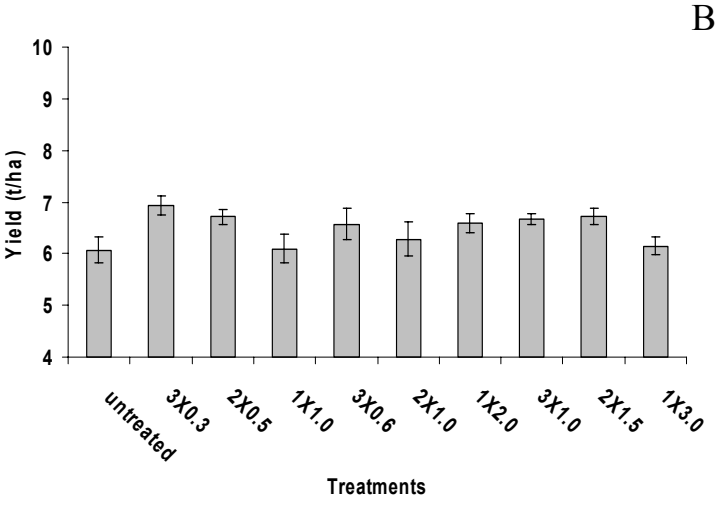


Figure 46. Effect of fungicide applications on Green Leaf Area and yield. GLA of Leaf 2 and flag at GS 75(A) and yields (B). Spray number and rates of Comet in l/ha are given for each treatment. Standard error bars are shown.





2.6.2 Effect of mixtures and alternations on QoI-resistance development

Location Terrington in 2003

The average R-allele frequency in *Septoria tritici* populations sampled from whole plants before spraying was approximately 25 % at GS 32. Sampling at GS 39 and 59, after application of two and three sprays, respectively, revealed that average R-allele frequencies had increased in plots treated with Comet as straight product, in alternation or in mixtures (Figure 47A). Low average frequencies between 14 and 39 % were measured in untreated plots and plots treated with Opus only. After four sprays (at GS 75), the lowest average R-allele frequencies, between 55 and 67 %, were found in the untreated and Opus treated plots (Figure 47B, C). Highest R-allele frequencies, between 99 and 100 %, were determined in plots that received four sprays of Comet at 0.25 l/ha. There was little difference in average R-allele frequencies on Leaf 3 and 2 for the two programmes with mixtures; only a relative low average R-allele frequency of 68 % was measured in the flag leaves sampled from plots treated with four sprays of mixtures with both Opus and Comet at 0.25 l/ha. Of the two alternation programmes, the sequence Comet-Opus-Comet-Opus resulted in lower average R-allele frequencies than Opus-Comet-Opus-Comet but there was a high level of variation in the results.

Disease assessments using PCR showed lower levels of disease on Leaf 3 after two sprays for both mixture programmes in comparison with the other treatments (Figure 48). After three sprays lowest disease levels were determined for both mixtures and the straight Opus programme. Assessment of the final disease levels at GS 75 showed with exception of the programme with four sprays of Comet at 0.25 l/ha reduced disease levels for all treatments in comparison with the untreated plots. The highest level of control was achieved with both mixture programmes.

Visual assessments of disease in the upper two leaves at GS 75 showed that disease levels were very low in all plots (Figure 49). All treatments resulted in lower levels of disease in comparison with the untreated plots. The highest average levels of disease were measured for the untreated plots with 7.8 and 2.9 % of disease present on Leaf 2 and the flag, respectively. Average disease levels of 4.4, 2.0 and 1.5 % on Leaf 2 were measured in plots treated with four sprays of Comet, four sprays of Opus and the alternation Opus-Comet-Opus-Comet, respectively. No disease was detected in the two upper leaves after treatment with mixtures.

In comparison with the untreated plots, all treatments increased significantly the GLA (Figure 50A), particularly for Leaf 2. The average GLA of Leaf 2 was 74 % in the untreated plots whereas values between 92 and 98 % were measured in the treated plots. None of the treatments resulted in a significant increase in yield with average yields between 11.1 and 11.7 recorded for all plots (Figure 50B).

Figure 47. Effect of fungicide applications on QoI resistance development in populations of *Septoria tritici*. R-allele frequencies for populations sampled from different leaf layers after two (A), three (B) and four sprays (C) at GS 39, 59 and 75, respectively. Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.

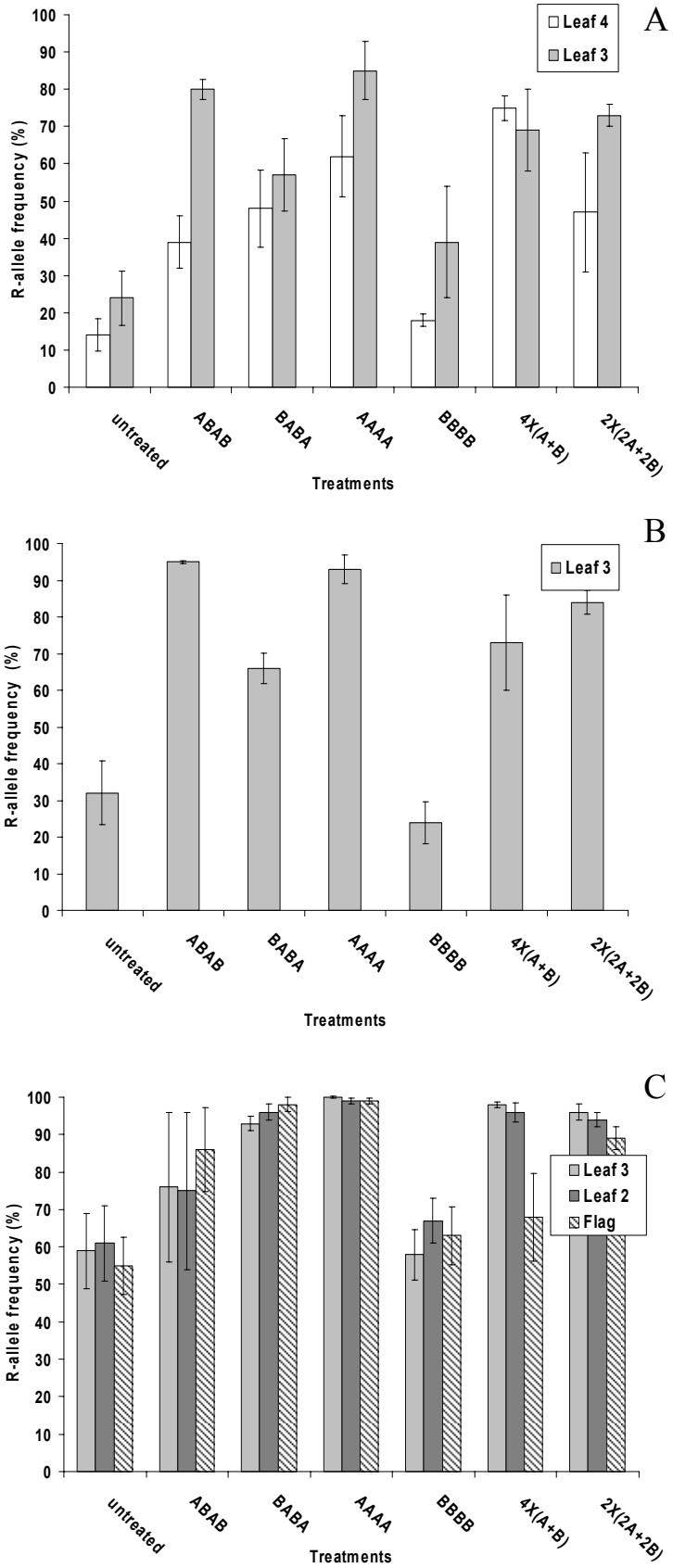


Figure 48. Effect of fungicide applications on Septoria infection levels as determined by PCR. Pathogen DNA levels in leaf samples after the 1st (A), 2nd (B) and 3rd spray (C) at GS 39, 59 and 75, respectively. Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.

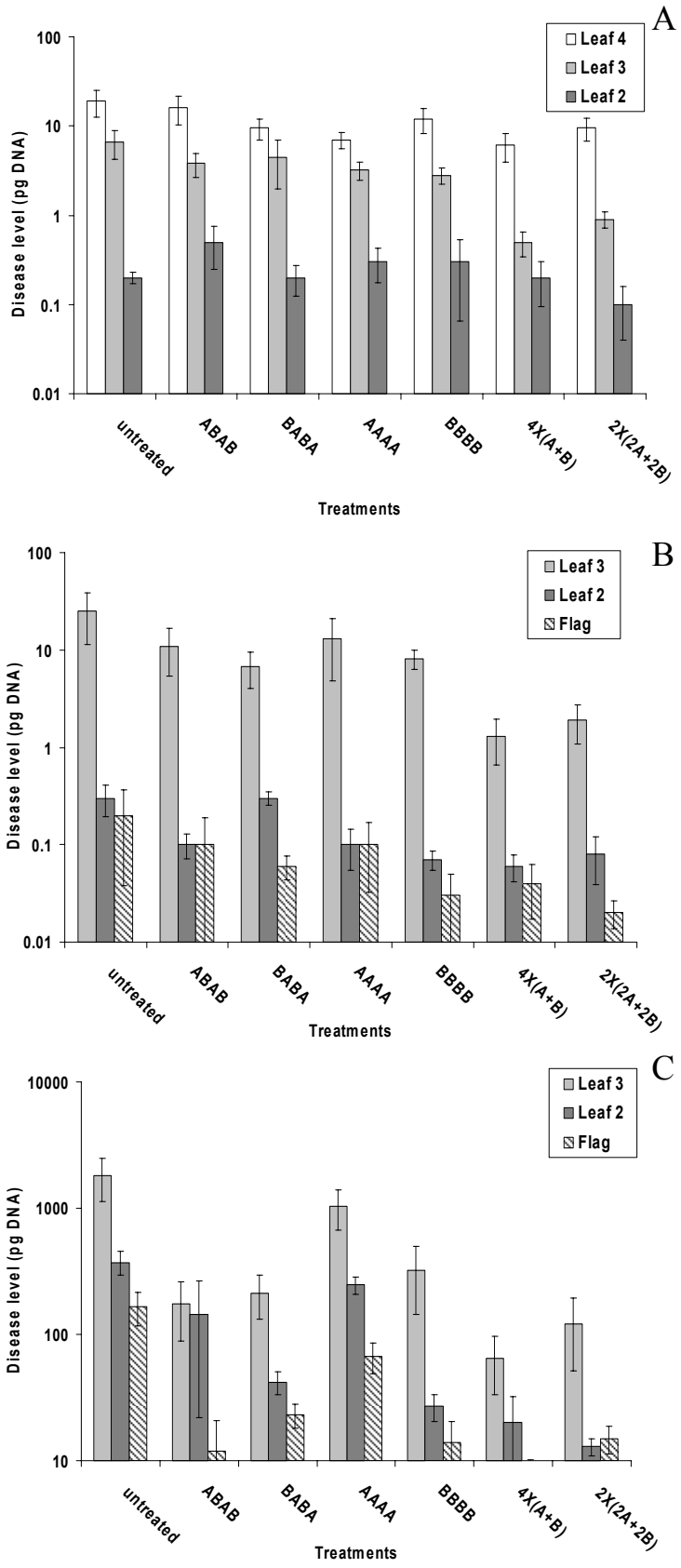


Figure 49. Effect of fungicide applications on Septoria disease levels as determined by visual assessments. Disease levels for Leaf 2 and flag at GS 75 are presented. Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.

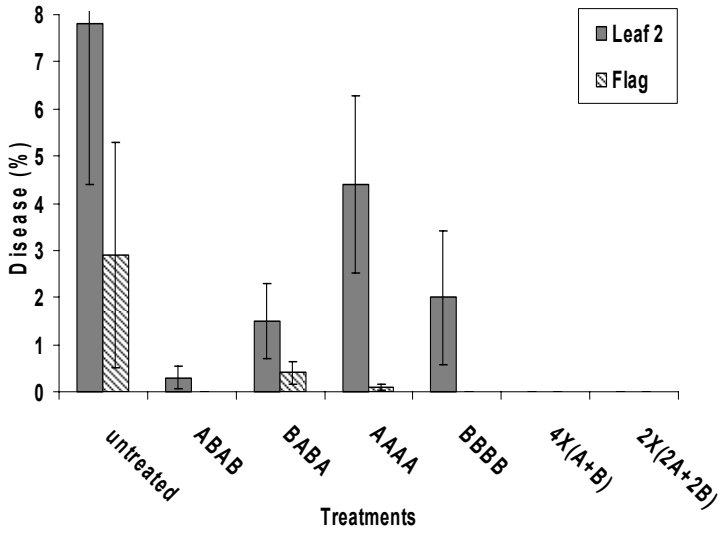
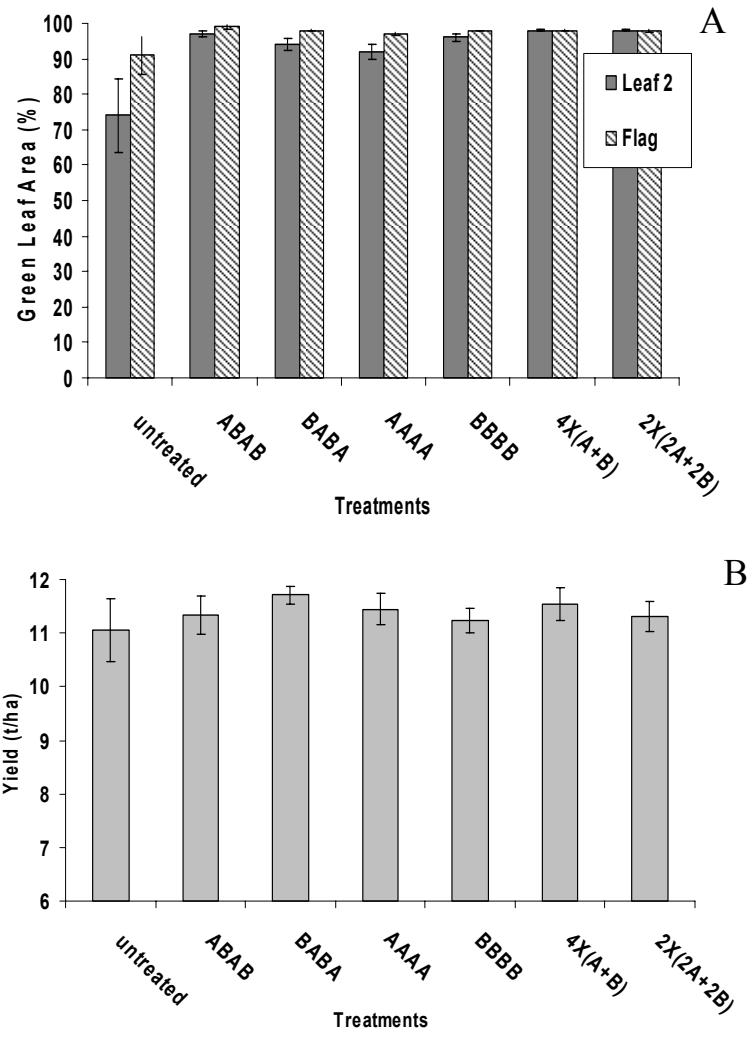


Figure 50. Effect of fungicide applications on Green Leaf Area and yield. GLA of Leaf 2 and flag at GS 75(A) and yields (B). Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.



Location Dundee in 2003

The average R-allele frequency in *Septoria tritici* populations sampled from whole plants at GS 32 before spraying was approximately 9 %. Sampling at GS 39 and 59, after the application of two and three sprays, respectively, revealed a significant increase in average R-allele frequencies in plots treated with Comet as a straight product, in alternation or in mixtures (Figure 51A). Low average frequencies between 10 and 19 % were measured in untreated plots and plots treated with Opus only. After four sprays (at GS 75), the lowest average R-allele frequencies, 10 and 16 % for Leaf 2 and flag leaf, respectively, were detected in the untreated plots (Figure 51B, C). The average R-allele frequencies were higher in the plots treated four times with Opus where values of 18 and 32 % for Leaf 2 and the flag leaf populations, respectively, were measured. R-allele frequencies between 63 and 97 % were measured for Leaf 2 and flag leaf populations sampled from the remaining plots, where Comet had been applied as either as a straight product, or in alternation or mixtures. In these treatments, the highest R-allele frequencies were detected in plots that had received four sprays of Comet at 0.25 l/ha, whereas the lowest frequencies were measured for the two spray mixture programme with both Opus and Comet at 0.5 l/ha.

Disease assessments by PCR showed lower levels of disease after two, three and four sprays on most leaf layers in treated plots, by comparison with untreated plots (Figure 52). After four sprays lowest disease levels were recorded for the four spray mixture programme with both Opus and Comet at 0.25 l/ha, whereas by comparison with the other treatments four sprays of Comet at 0.25 l/ha was the least effective treatment .

Visual assessments of disease on the upper two leaves at GS 75 showed that all fungicide treatments resulted in lower levels of disease by comparison with the untreated plots (Figure 53). The highest average levels of disease were measured for the untreated plots with 13.8 and 3.5 % of disease present on Leaf 2 and the flag leaf, respectively. Average disease levels on Leaf 2 and the flag leaf were very similar for all fungicide programmes with negligible amounts present on the flag, and between 1.5 and 2.5 % leaf area affected on Leaf 2.

All treatments significantly increased GLA by comparison with the untreated plots, (Figure 54), particularly on Leaf 2. The average GLA of Leaf 2 was 61 % in untreated plots whereas values between 91 and 95 % were measured in treated plots.

Figure 51. Effect of fungicide applications on QoI resistance development in populations of *S. tritici*. R-allele frequencies for populations sampled from different leaf layers after two (A), three (B) and four sprays (C) at GS 39, 59 and 75, respectively. Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.

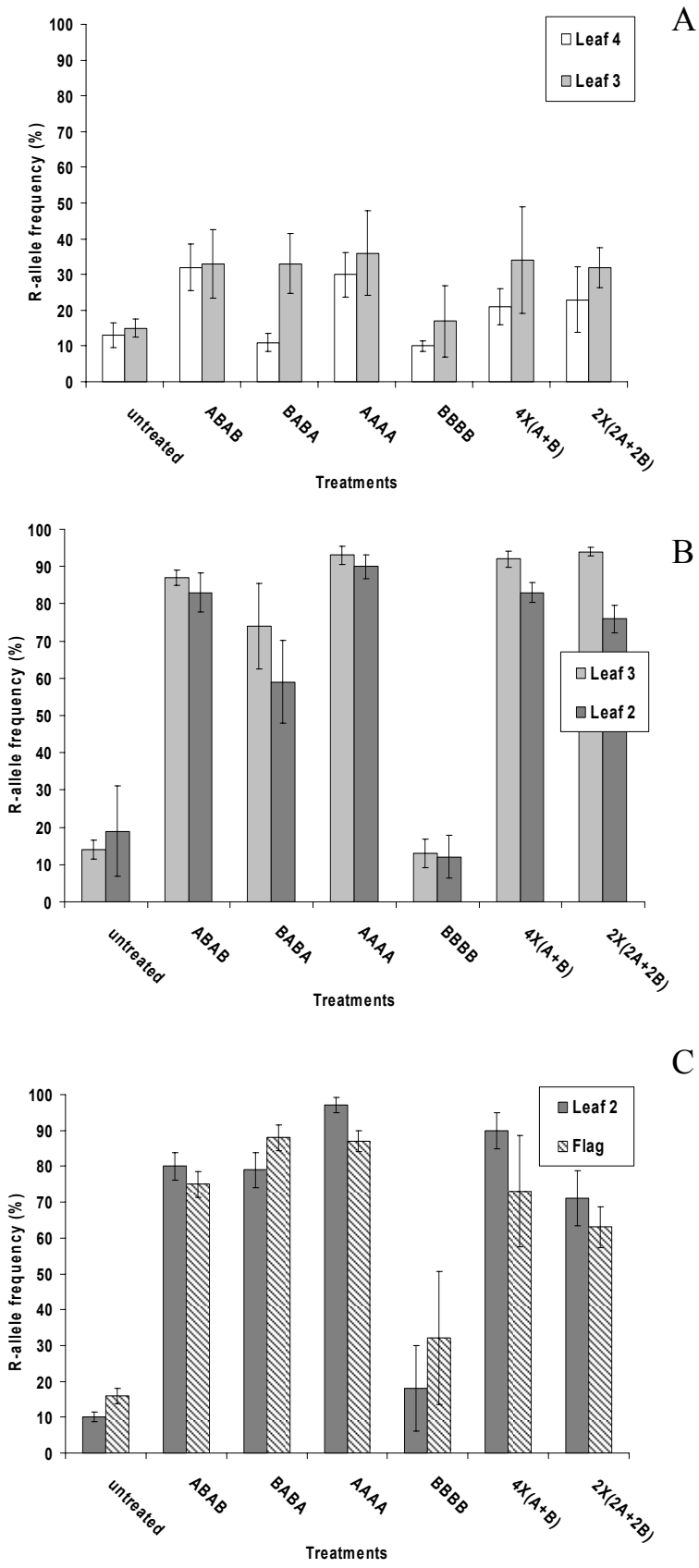


Figure 52. Effect of fungicide applications on Septoria infection levels as determined by PCR. Pathogen DNA levels in leaf samples after the 1st (A), 2nd (B) and 3rd spray (C) at GS 39, 59 and 75, respectively. Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.

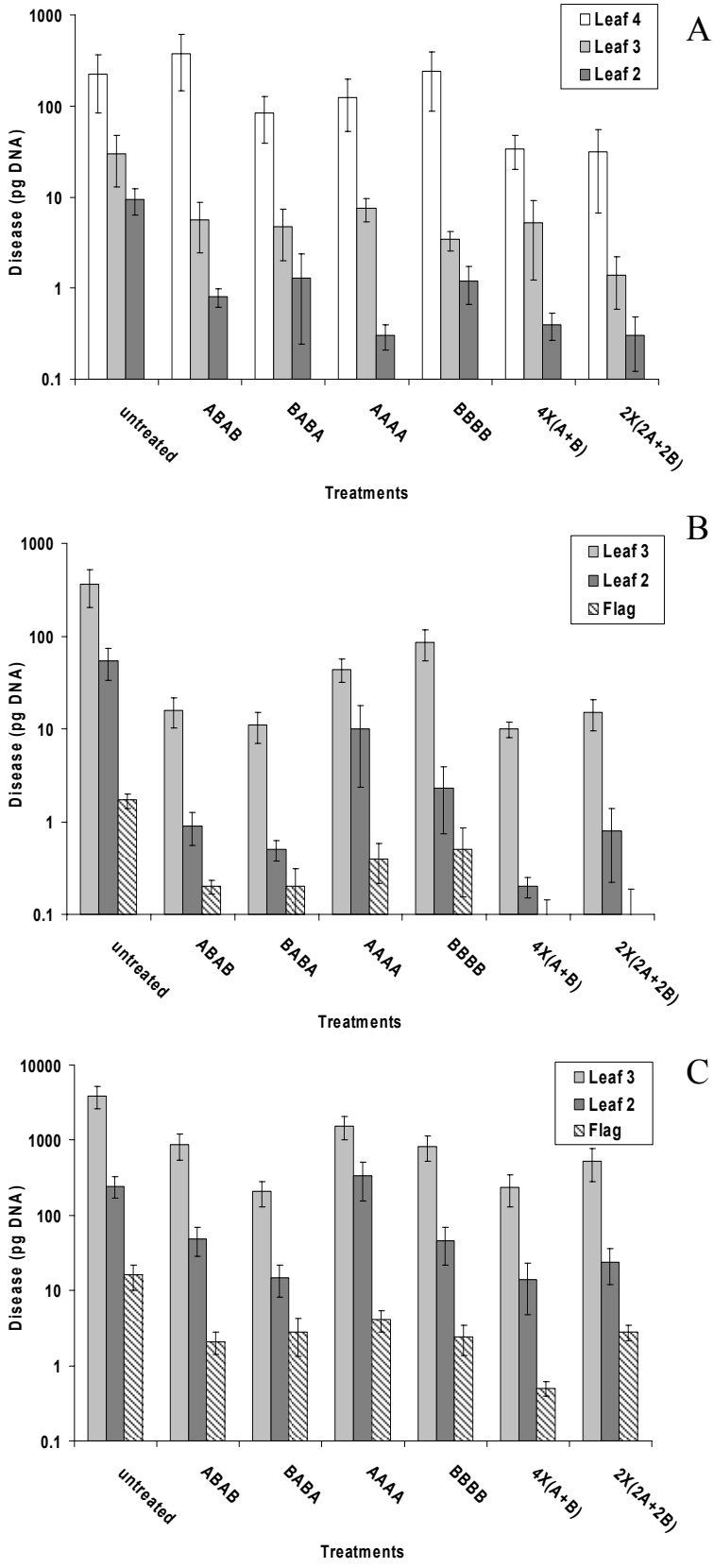


Figure 53. Effect of fungicide applications on Septoria disease levels as determined by visual assessments. Disease levels for on Leaf 2 and flag at GS 75 are presented. Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.

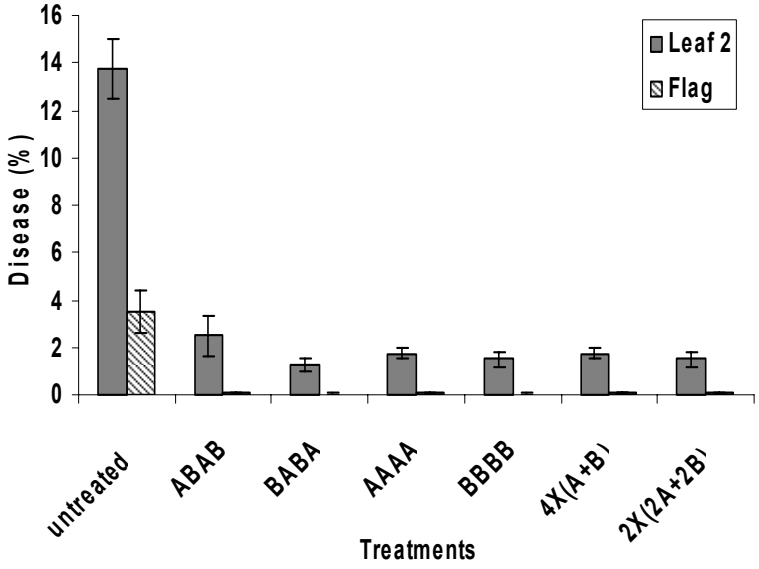
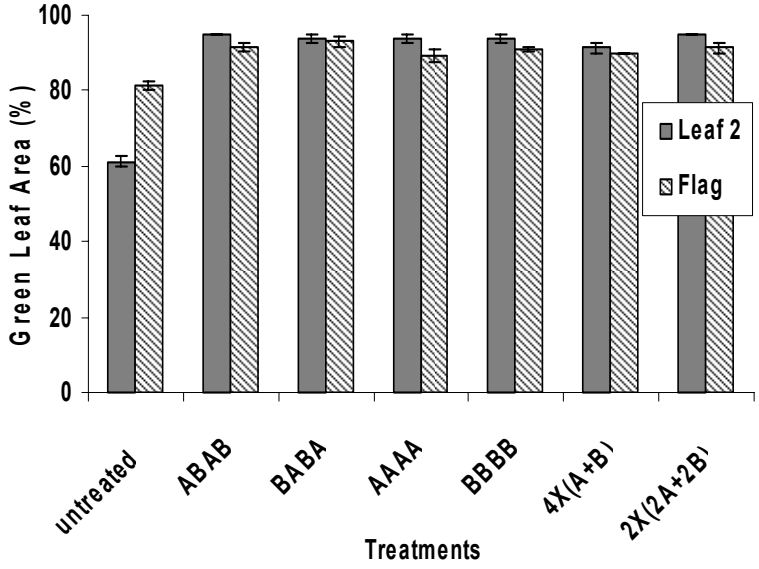


Figure 54. Effect of fungicide applications on Green Leaf Area of upper leaves at GS 75. Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.



Location Terrington in 2004

The average R-allele frequency in *S. tritici* populations sampled from whole plants before spraying was approximately 76 % at GS 32. Sampling at GS 39, after the application of two sprays, showed increased R-allele frequencies between 91 and 100% in leaf populations sampled from plots treated with Comet as a straight product, in alternation or in mixtures (Figure 55A). The average R-allele frequencies in untreated and Opus treated plots remained lower, between 74 and 82 %. The average R-allele frequencies measured after the third and fourth sprays were very similar to the frequencies measured after the second spray (Figure 55B, C). The final average R-allele frequencies in the untreated plots were 77, 81 and 84 % for Leaf 3, Leaf 2 and flag leaf populations, respectively.

Disease assessments by PCR showed lower levels of disease after two, three and four sprays on most leaf layers of the treated plots by comparison with the untreated plots (Figure 56). After four sprays, the lowest disease levels were found in the four spray mixture programme with both Opus and Comet at 0.25 l/ha, whereas by comparison with other treatments four sprays of Comet at 0.25 l/ha was clearly the least effective treatment. The alternation sequence Comet-Opus-Comet-Opus was more effective than the sequence Opus-Comet-Opus-Comet.

Visual assessments of disease on the upper two leaves at GS 75 showed that disease levels were high in all plots (Figure 57). All treatments resulted in lower levels of disease by comparison with the untreated plots in which 64 and 39 % of disease was present on Leaf 2 and the flag leaf, respectively. Of the different treatments four sprays of Comet was least effective with average disease levels of 58 and 19 % present on Leaf 2 and the flag leaf, respectively. Highest levels of disease control were achieved with mixtures of Comet and Opus.

All treatments significantly increased GLA of the two upper leaves bn comparison with untreated plots (Figure 58A). Highest average GLA increases were measured for the mixture and solo Opus programmes, while the lowest increase was measured for four sprays of Comet. Of the alternation programmes, the sequence Comet-Opus-Comet-Opus resulted in slightly higher levels of GLA. Average GLA correlated well with the overall yields. The highest average yield, 9.6 t/ha, was obtained with four sprays of the mixture Opus and Comet, both at 0.25 l/ha, whereas the yield of the untreated plots was only 6.7 t/ha (Figure 58B).

Figure 55. Effect of fungicide applications on QoI resistance development in populations of *S. tritici*. R-allele frequencies for populations sampled from different leaf layers after two (A), three (B) and four sprays (C) at GS 39, 59 and 75, respectively. Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.

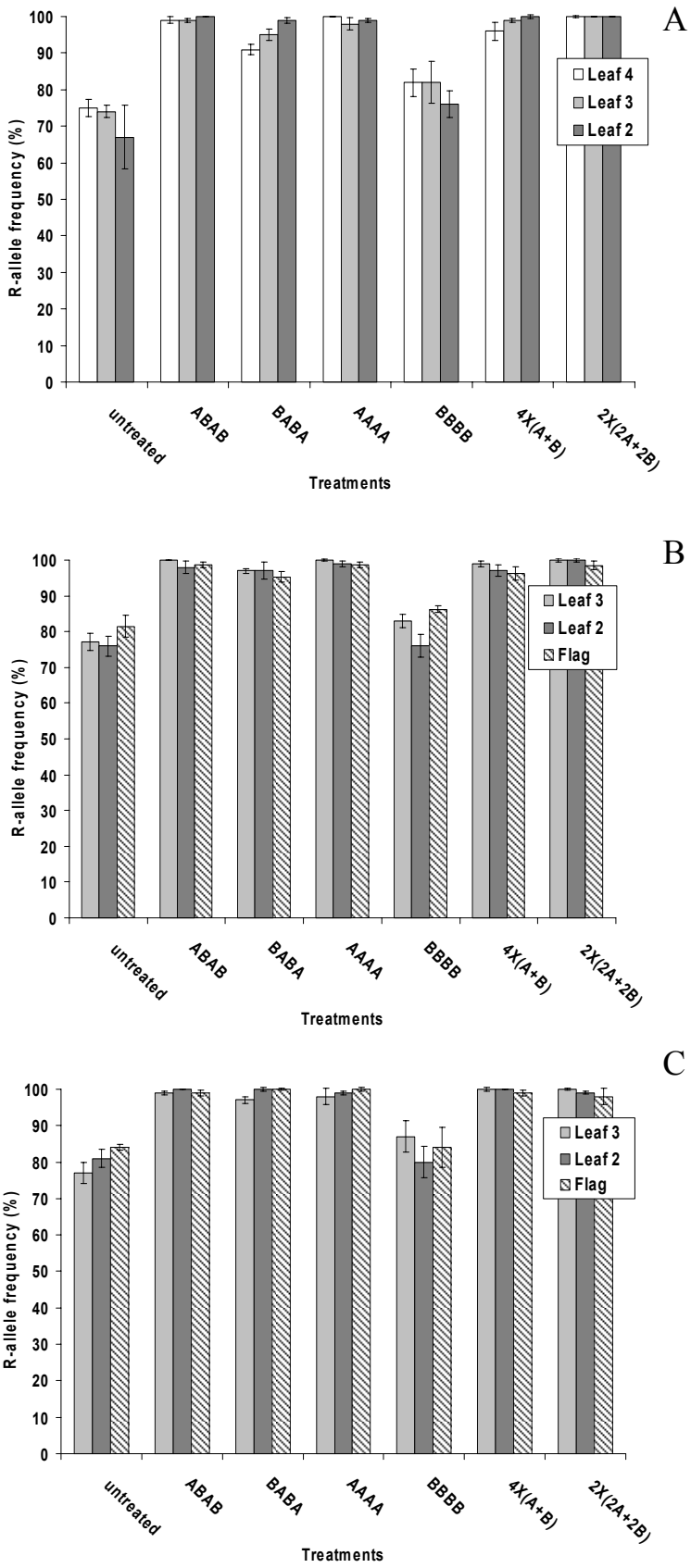


Figure 56. Effect of fungicide applications on Septoria infection levels as determined by PCR. Pathogen DNA levels in leaf samples after the 1st (A), 2nd (B) and 3rd spray (C) at GS 39, 59 and 75, respectively. Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.

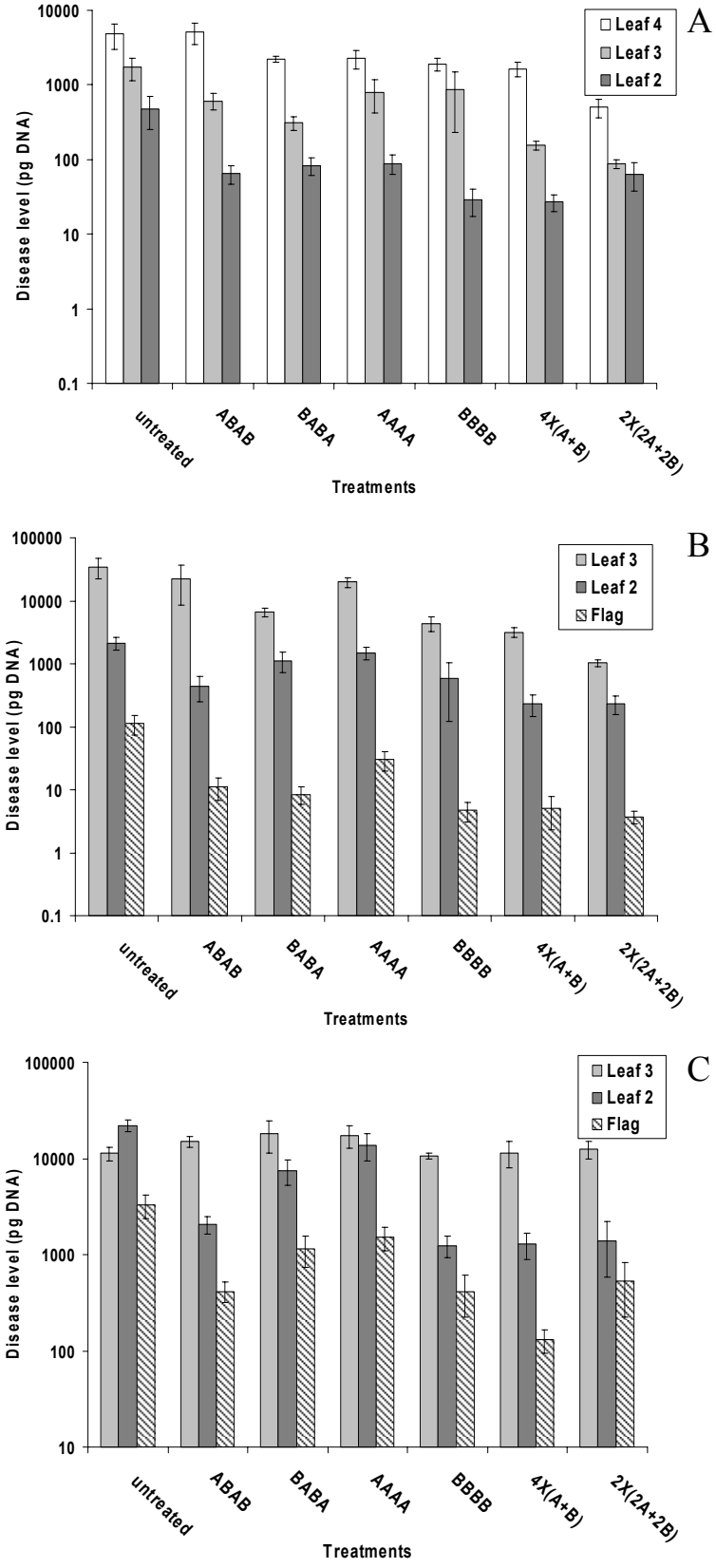


Figure 57. Effect of fungicide applications on Septoria disease levels as determined by visual assessments. Disease levels for Leaf 2 and flag at GS 75 are presented. Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.

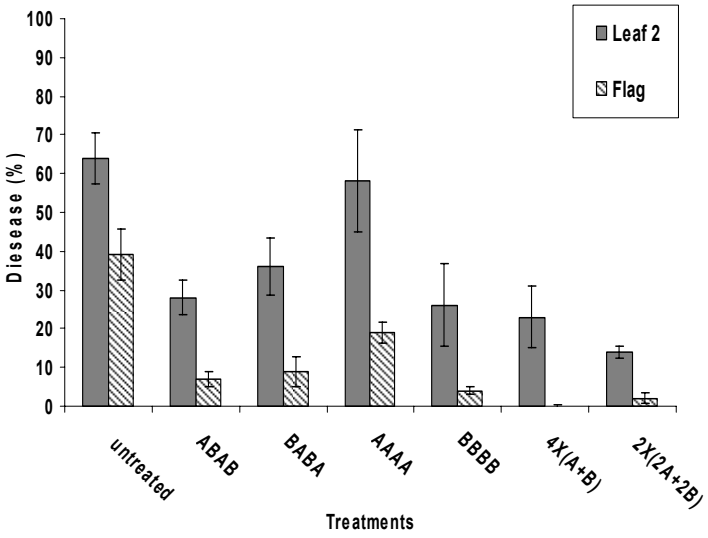
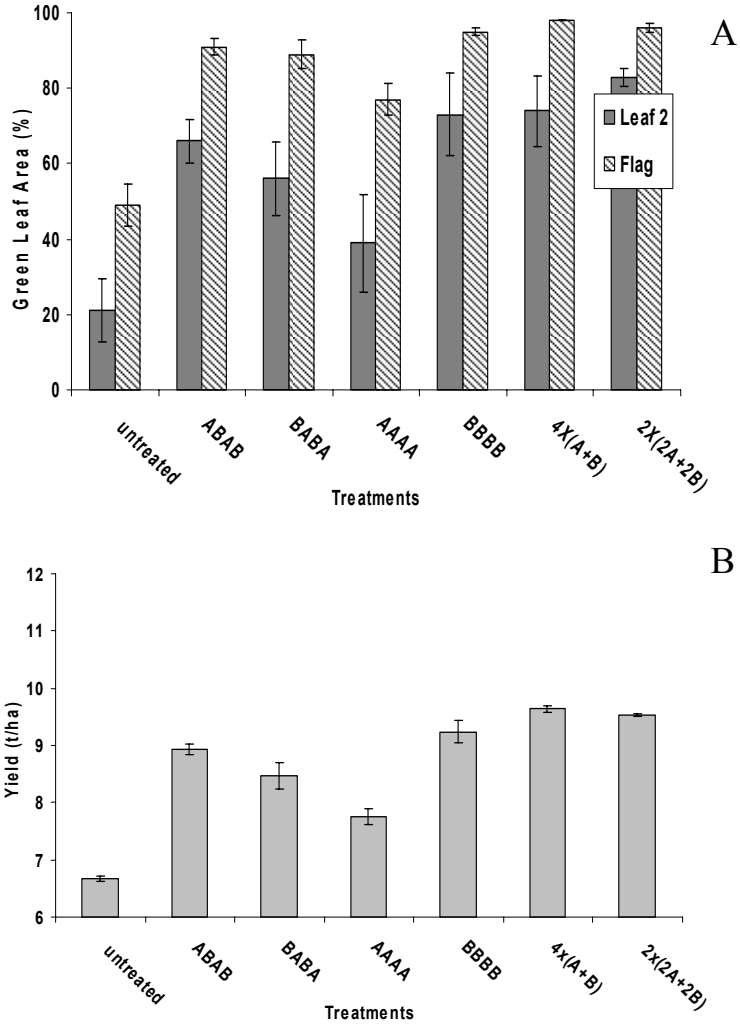


Figure 58. Effect of fungicide applications on Green Leaf Area and yield. GLA of Leaf 2 and flag at GS 75(A) and yields (B). Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.



Location Edinburgh in 2004

The average R-allele frequency was 26 % before spraying at GS 32. Sampling at GS 39, after application of two sprays, detected increased R-allele frequencies between 61 and 99 % for leaf populations sampled from plots treated with Comet as a straight product, in alternation or in mixtures (Figure 59A). The average R-allele frequencies in the untreated plots remained low during the season between 24 and 40 %. After the third and fourth fungicide applications, the average R-allele frequencies further increased in the fungicide treated plots (Figure 59B, C). Highest R-allele frequencies, between 98 and 100 %, were measured in populations sampled from the plot that had received four sprays of Comet at 0.25 l/ha. The average R-allele frequencies from the remaining plots that had received Comet were also very high, whereas frequencies between 36 and 75 % were measured for populations sampled from Opus treated plots.

Disease assessments by PCR showed lower levels of disease after two, three and four sprays on all leaves sampled from treated plots by comparison with untreated plots (Figure 60). After four sprays, lowest disease levels were determined for the four spray mixture programme with both Opus and Comet at 0.25 l/ha, whereas four sprays of Comet at 0.25 l/ha was clearly least effective by comparison with the other treatments.

Visual assessments of disease on the upper two leaves at GS 75 showed that disease was very low on Leaf 2 and mostly absent on the flag leaf (Figure 61). In untreated plots an average disease level of 4.5 % was measured on Leaf 2, whereas disease levels in treated plots were lower, between 1.1 and 1.4 %.

By comparison with the untreated plots, only the GLA of Leaf 2 was slightly increased by up to 6 % in treated plots (Figure 62A). There were no differences between the different treatments with regard to GLA of the two upper leaves. By comparison with the untreated and Opus treated plots, treatments containing Comet sprays only marginally increased the yield up to 0.6 t/ha (Figure 62B).

Figure 59. Effect of fungicide applications on QoI resistance development in populations of *S. tritici*. R-allele frequencies for populations sampled from different leaf layers after two (A), three (B) and four sprays (C) at GS 39, 59 and 75, respectively. Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.

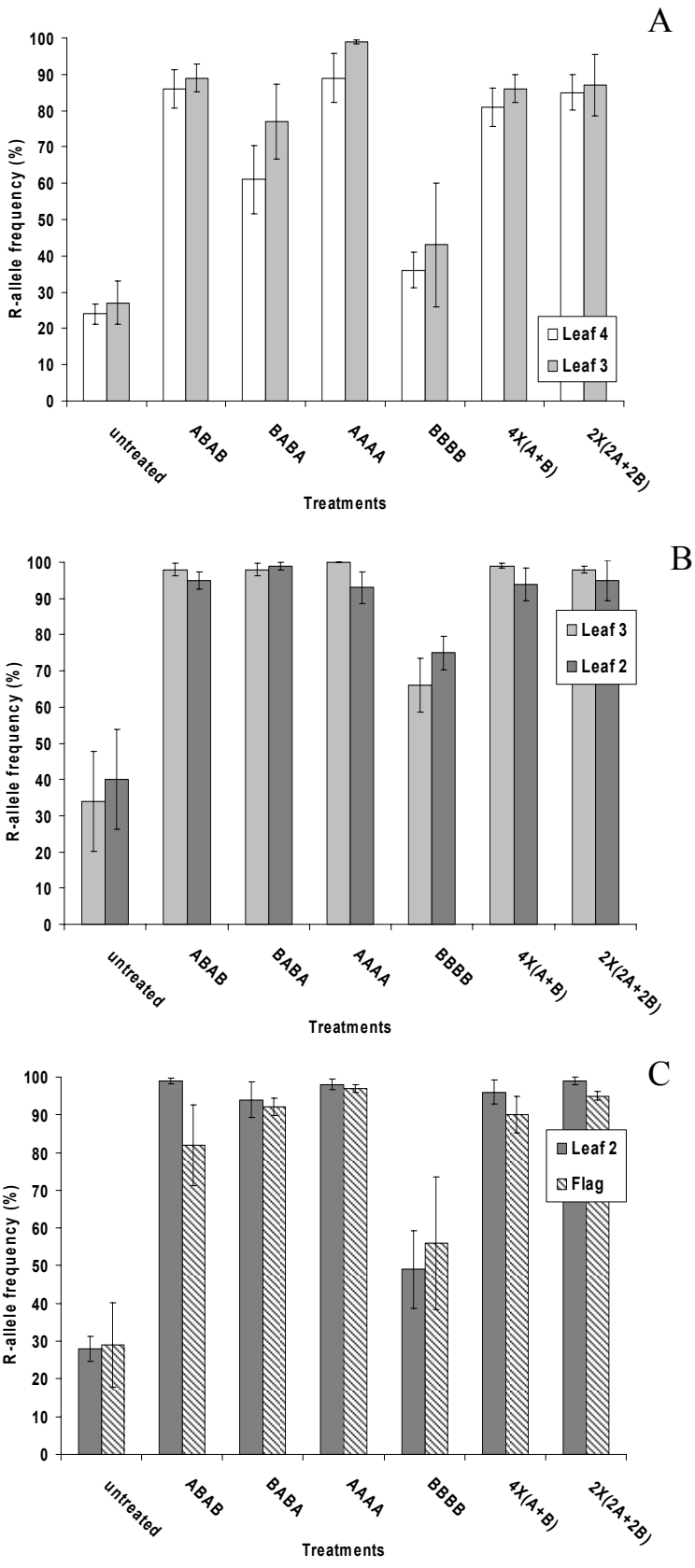


Figure 60. Effect of fungicide applications on Septoria infection levels as determined by PCR. Pathogen DNA levels in leaf samples after the 1st (A), 2nd (B) and 3rd spray (C) at GS 39, 59 and 75, respectively. Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.

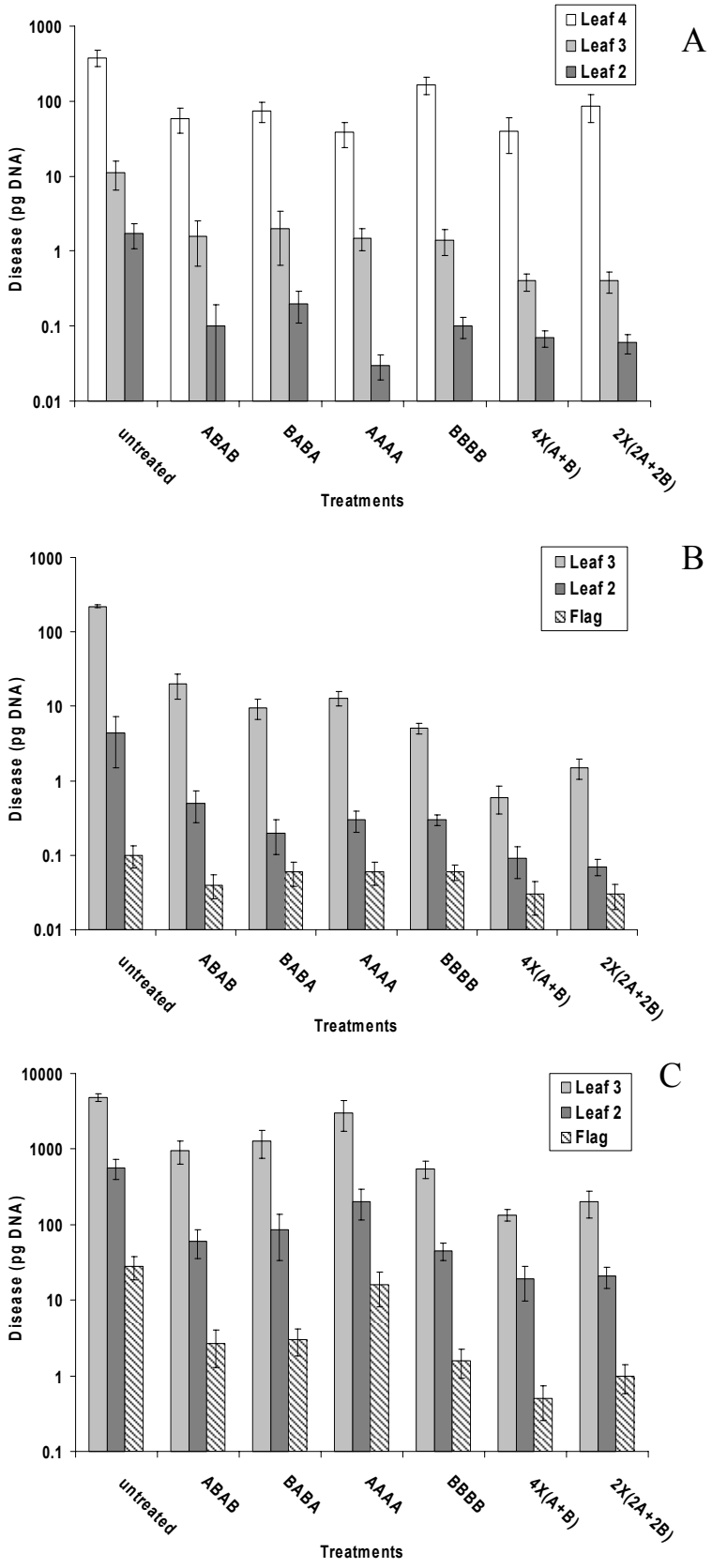


Figure 61. Effect of fungicide applications on Septoria disease levels as determined by visual assessments. Disease levels for Leaf 2 and flag at GS 75 are presented. Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.

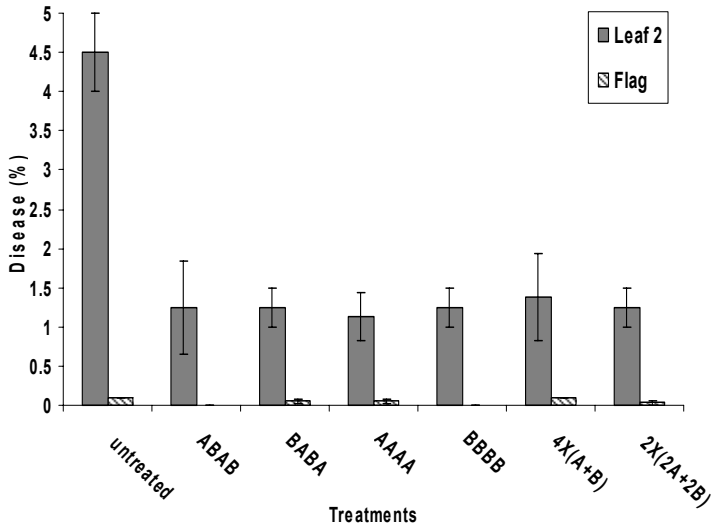
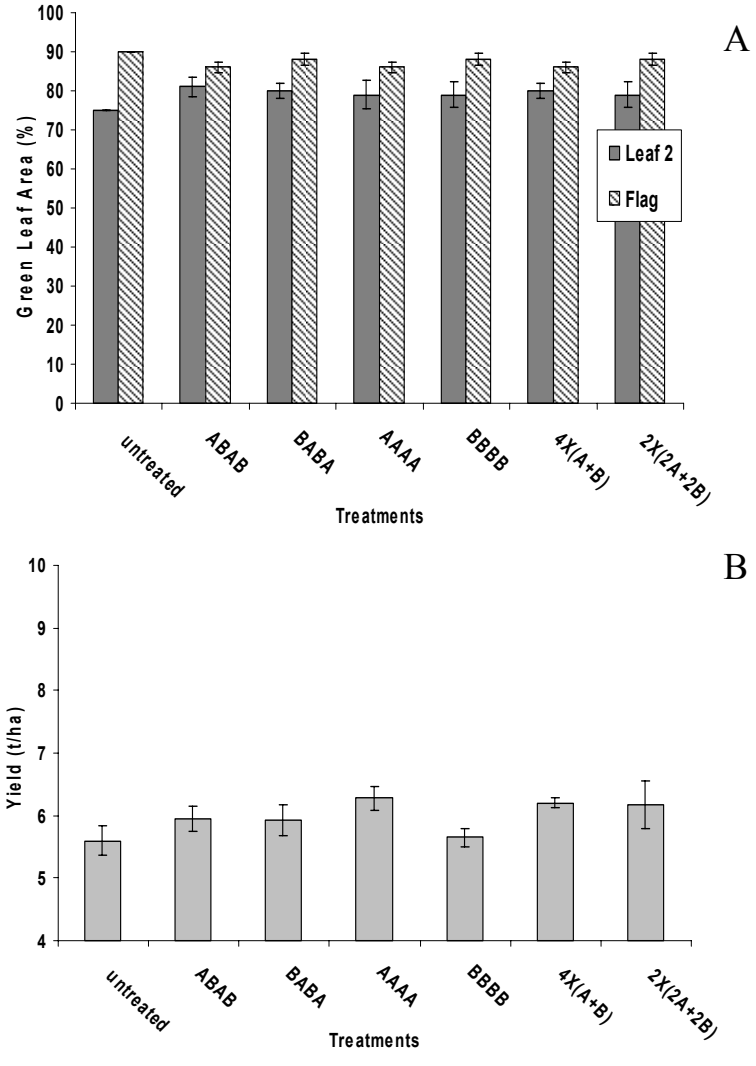


Figure 62. Effect of fungicide applications on Green Leaf Area and yield. GLA of Leaf 2 and flag at GS 75(A) and yields (B). Treatments: A, Comet at 0.25 l/ha; B, Opus at 0.25 l/ha. Standard error bars are shown.



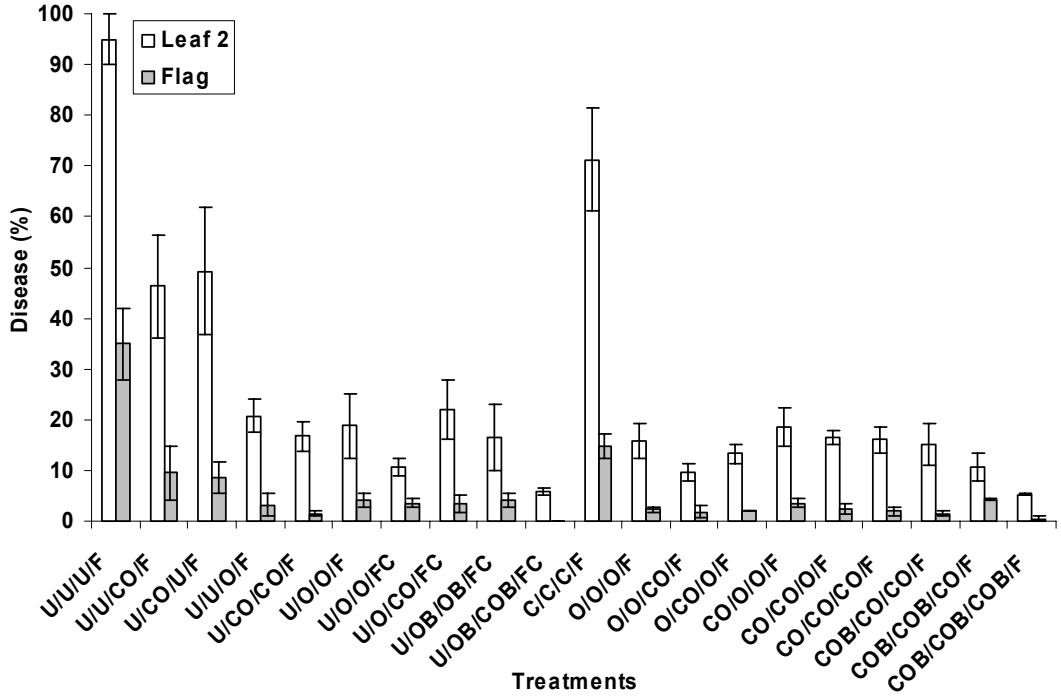
2.7 Septoria core field experiment in 2005

Due to the rapid build-up of QoI resistance during the 2002/03 and 2003/04 growing seasons, with average R-allele frequencies rising from 28 to 88%, respectively, it was anticipated that during the 2004/05 growing season the whole UK population of *S. tritici* would, for all practical purposes, be QoI resistant. It was therefore decided to focus more on disease control, to determine how this might be compromised and yields affected when using QoI fungicides in both protectant and eradicant situations, and to compare efficacy with alternative triazole and chlorothalonil treatments.

Location Whitesome in 2005

Visual assessment data presented in Figure 63 show disease levels in different plots at GS 75 after fungicide application. By comparison with untreated plots, highest average disease levels were observed in plots that had received a single spray of the Comet /Opus mixture at T1 or T2, or three sprays of Comet.

Figure 63. Effect of fungicide applications on Septoria disease levels as determined by visual assessments. Disease levels for Leaf 2 and flag at GS 75 are presented. Treatments: U, untreated; F, Folicur (a.i. tebuconazole); C, Comet (a.i. pyraclostrobin); O, Opus (a.i. epoxiconazole); B, Bravo (a.i. chlorothalonil); CO, mixture of Comet and Opus; OB, mixture of Opus and Bravo; COB, mixture of Comet, Opus and Bravo; FC, mixture of Comet and Folicur. Sprays applied at GS 30, 32, 39 and 59. Rates of straight products and mixture components are given in Table 6. Standard error bars are shown.

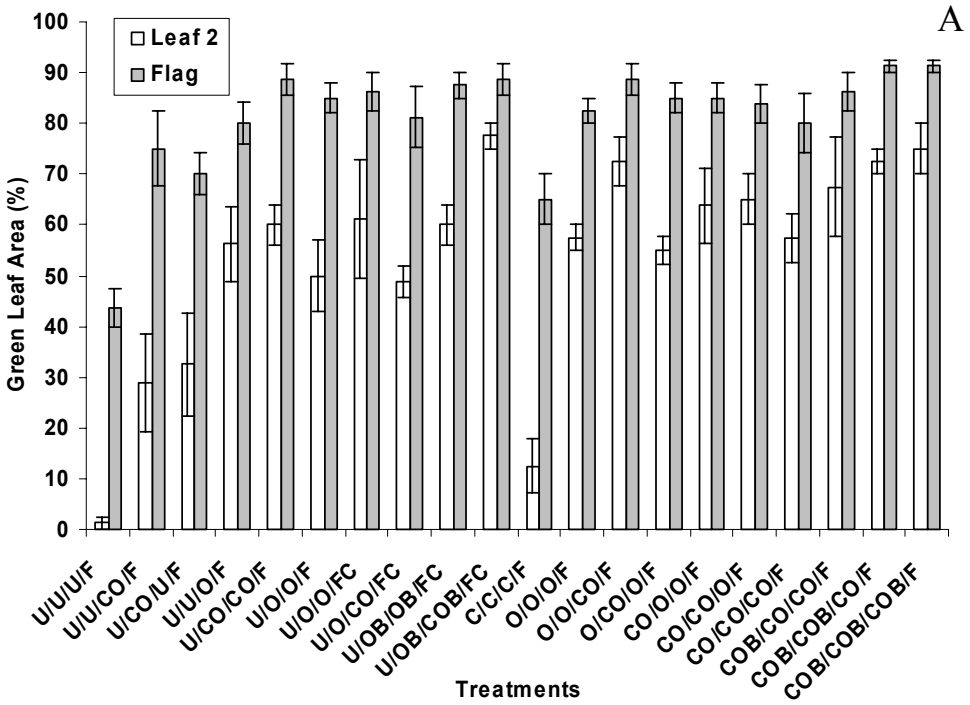


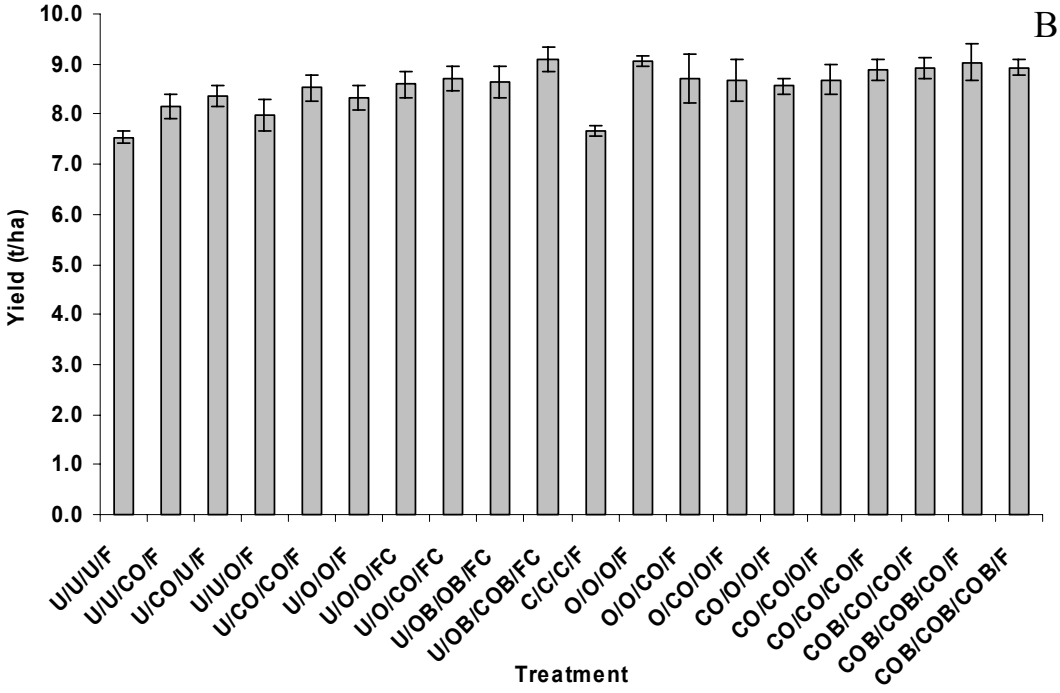
Disease control was clearly compromised by QoI resistance as a single spray of Opus at T2, with 20.8 and 3.3 % of disease recorded for leaf 2 and the flag leaf, respectively, was much more effective than three sprays of Comet at T0, T1 and T2 where 71.3 and 14.8 % disease was recorded for Leaf 2 and the flag leaf,

respectively. The average disease level on Leaf 2 and the flag leaf sampled from untreated plots was 95 and 35 %, respectively. For all other treatments, mostly based on multiple sprays and/or mixture applications, much lower levels of disease were recorded for the two upper leaves. PCR testing of leaf samples collected from one randomised block containing all different treatments showed lower Septoria infection levels on Leaf 2 for most samples that had received four sprays in comparison with samples that had received three sprays; this trend was not observed with visual assessments. As expected, the R-allele frequencies measured for populations in these samples were all between 95 and 100%.

Green leaf area assessments at GS 75 showed a clear link between the average level of disease and GLA with lowest levels of GLA measured for untreated, three sprays of Comet (T0, T1 and T2) and single mixture spray applications of comet with Opus at T1 and T2 (Figure 64A). In comparison with other treatments, average GLA levels for Leaf 2 were especially low for these plots with 1.3, 12.5, 28.8 and 32.5 % for the untreated, three sprays of Comet (T0, T1 and T2) and single spray applications of the Comet /Opus mixture at T2 and T1. For plots that had received a T3 spray of Folicur, the highest Leaf 2 GLA level, 75 %, was measured with three sprays of the Comet/Opus/ Bravo mixture applied at T0, T1 and T2.

Figure 64. Effect of fungicide applications on Green Leaf Area (A) and yield (B). Treatments: U, untreated; F, Folicur (a.i. tebuconazole); C, Comet (a.i. pyraclostrobin); O, Opus (a.i. epoxiconazole); B, Bravo (a.i. chlorothalonil); CO, mixture of Comet and Opus; OB, mixture of Opus and Bravo; COB, mixture of Comet, Opus and Bravo; FC, mixture of Comet and Folicur. Sprays applied at GS 30, 32, 39 and 59. Rates of straight products and mixture components are given in Table 6. Standard error bars are shown.



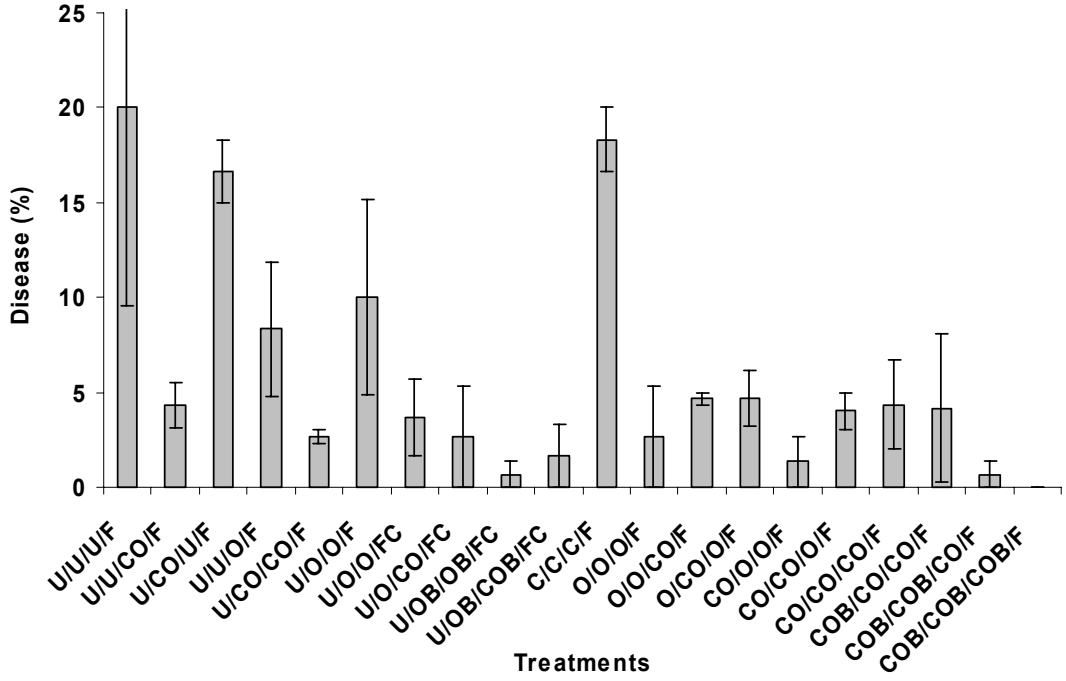


Yield data showed that high levels of disease and low levels of GLA were generally linked with yield loss (Figure 64B). Lowest average yields were recorded in untreated plots and plots treated three times with Comet at T0, T1 and T2, with yields of 7.54 and 7.67 t/ha, respectively. The yield of the untreated plot was reduced by approximately 1.5 t/ha by comparison with the highest yielding treatments. Interestingly, despite suboptimal levels of GLA on the upper leaves, one of the highest average yields, 9.05 t/ha, was measured in plots treated with three sprays of Opus at T0, T1 and T2.

Location Terrington in 2005

Visual assessment data presented in Figure 65 show disease levels in different plots at GS 77 after fungicide application. Because Leaf 2 was senesced in the untreated plots and in some of the treated plots only disease levels for the flag are given. By comparison with untreated plots (T3 spray of Folicur only), highest average disease levels were observed in plots that had received a single spray of the Comet /Opus mixture at T1 or three sprays of Comet at T0, T1 and T2. Disease control was clearly compromised by QoI resistance as a single spray of Opus at T2, with an average disease level of 8.3 % on the flag, was much more effective than three sprays of Comet at T0, T1 and T2 where 18.3 % of disease was recorded for the flag. The average disease level on the flag leaf sampled from untreated plots was 20 %. For all other treatments, mostly based on multiple sprays and/or mixture applications, much lower levels of disease were recorded for the flag leaves. Disease was absent on flags sampled from plots that had received three sprays of the Comet/Opus/Bravo mixture.

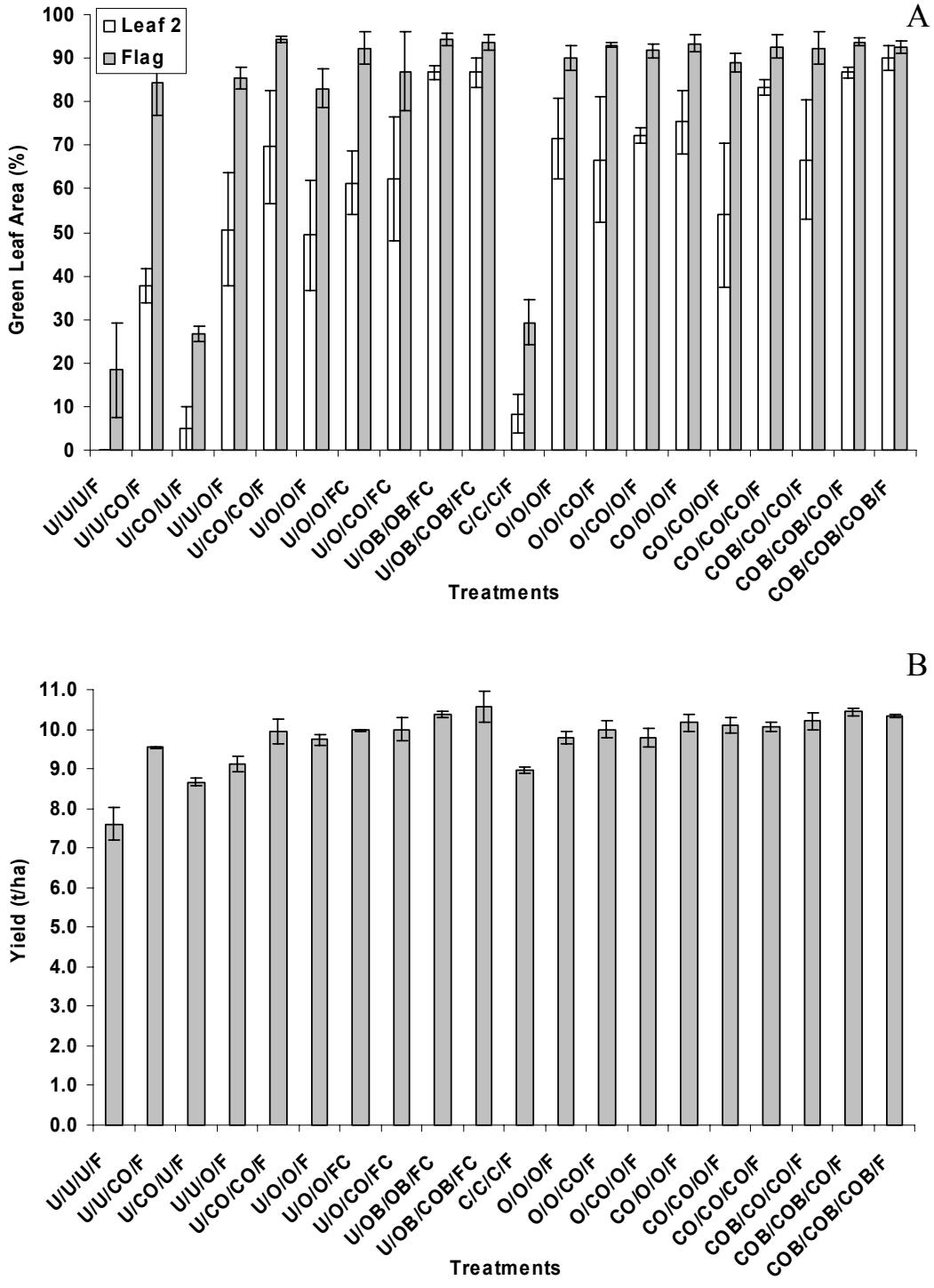
Figure 65. Effect of fungicide applications on Septoria disease levels as determined by visual assessments. Disease levels for the flag at GS 77 are presented. Treatments: U, untreated; F, Folicur (a.i. tebuconazole); C, Comet (a.i. pyraclostrobin); O, Opus (a.i. epoxiconazole); B, Bravo (a.i. chlorothalonil); CO, mixture of Comet and Opus; OB, mixture of Opus and Bravo; COB, mixture of Comet, Opus and Bravo; FC, mixture of Comet and Folicur. Sprays applied at GS 30, 32, 39 and 59. Rates of straight products and mixture components are given in Table 6. Standard error bars are shown.



Green leaf area assessments at GS 77 showed a clear link between the average level of disease and GLA with lowest levels of GLA measured for untreated, three sprays of Comet (T0, T1 and T2) and a single mixture spray applications of Comet with Opus at T1 (Figure 66A). In comparison with other treatments, average GLA levels for the flag were especially low for these plots with 18.3, 29.3 and 26.7 % for the untreated, three sprays of Comet (T0, T1 and T2) and single spray applications of the Comet/Opus mixture at T1, respectively. For plots that had received a T3 spray of Folicur, the highest Leaf 2 GLA level, 90 %, was measured for plots treated with three sprays of the Comet/Opus/ Bravo mixture at T0, T1 and T2.

Yield data showed that high levels of disease and low levels of GLA were linked with yield loss (Figure 66B). Lowest average yields were recorded in untreated plots (T3 spray of Folicur only), plots treated three times with Comet at T0, T1 and T2, plots treated with a single spray of the Comet/Opus mixture at T1 and plots treated with a single Opus spray at T2, with yields of 7.60, 8.97, 8.67 and 9.13 t/ha, respectively. The average yield of the untreated plot was reduced by approximately 3.0 t/ha by comparison with the highest yielding fungicide programme consisting of a T1 application of Opus/Bravo mixture, T2 application of Comet/Opus/Bravo mixture and T3 application of the Folicur/Comet mixture. High yields >10.2 t/ha were obtained with all treatments containing mixtures with Opus and Bravo.

Figure 66. Effect of fungicide applications on Green Leaf Area (A) and yield (B). Treatments: U, untreated; F, Folicur (a.i. tebuconazole); C, Comet (a.i. pyraclostrobin); O, Opus (a.i. epoxiconazole); B, Bravo (a.i. chlorothalonil); CO, mixture of Comet and Opus; OB, mixture of Opus and Bravo; COB, mixture of Comet, Opus and Bravo; FC, mixture of Comet and Folicur. Sprays applied at GS 30, 32, 39 and 59. Rates of straight products and mixture components are given in Table 6. Standard error bars are shown.



2.8 QoI resistance development in other cereal pathogens

All industry partners participating in this project are continuously monitoring the status of QoI resistance in other fungal pathogens under the umbrella of the QoI Working Group of FRAC (Fungicide Resistance Action Committee). These surveys include cereal diseases (e.g. powdery mildew, Septoria leaf blotch, brown rust, net blotch, Rhynchosporium and tan spot), vine diseases (downy mildew and powdery mildew), pome fruit diseases (apple scab), potato/tomato diseases (late blight and early blight), and soybean diseases (Asian rust). The current resistance status of each disease is given and specific crop/pathogen guidelines are issued. With regard to cereal diseases, the F129L mutation has recently been detected in net blotch populations, and both F129L and G143A in tan spot populations. The implications of these developments are discussed. The latest report of the QoI Working Group is included in Appendix B. Additionally, Syngenta has compiled an extensive report from their studies on QoI resistance development in *Septoria tritici* populations which includes results of field trials carried out in the UK and mainland Europe (see Appendix C).

ACKNOWLEDGEMENTS

Many workers at each of the partner organisations participated in this project. We are grateful to all of them. Particular thanks are due to Juliet Motterram, Rothamsted Research, who processed most of the samples. Cliodhna McCartney (Queen's University of Belfast) is also gratefully acknowledged for collecting and testing Septoria leaf blotch samples from Northern Ireland.

PROJECT OUTPUT

Publications

- Fraaije BA, Lucas JA, Clark WS & Burnett FJ (2003) QoI resistance development in populations of cereal pathogens in the UK' In: Proceedings BCPC International Congress of Crop Science and Technology, November, BCPC: Alton, Hampshire, UK, pp 689-694 (on CD)
- Lucas, J. A. (2003). Resistance to QoI fungicides: implications for cereal disease management in Europe. *Pesticide Outlook* 14, 268-270.
- Fraaije BA & Lucas JA (2004) 'Evolution of QoI resistance in populations of *Mycosphaerella graminicola*' *Phytopathology* 94, 6, June (supplement), S31.
- Fraaije BA, Cools HJ, Fountaine J, Lovell DJ, Motteram J, West JS & Lucas JA (2005) QoI resistant isolates of *Mycosphaerella graminicola* and the role of ascospores in further spread of resistant alleles in field populations. *Phytopathology* 95, 933-941 (on CD)
- Fraaije BA, Burnett FJ, Clark WS, Motteram J & Lucas JA. Resistance development to Qo Inhibitors in populations of *Mycosphaerella graminicola* (2005) In: Modern fungicides and antifungal compounds IV, eds Dehne HW, Gisi U, Kuck K-H, Russell P and Lyr H. BCPC: Alton, Hampshire, UK, Chapter 8, pp 63-71 (on CD)
- Lucas JA (2005) QoI resistance in cereal pathogens: the European experience. *Phytopathology* 95, 6 S143.

Presentations

- Fraaije BA (2003) 'QoI resistance development in populations of cereal pathogens in the UK' In: Proceedings BCPC International Congress of Crop Science and Technology, November 10-12, Glasgow.
- Fraaije BA (2004) 'Resistance development to Qo Inhibitors in populations of *Mycosphaerella graminicola*' 14th International Symposium on Modern Fungicides and Antifungal Compounds, April 26-29, Reinhardsbrunn, Germany.
- Fraaije BA (2004) 'Evolution of QoI resistance in populations of *Mycosphaerella graminicola*' American Phytopathological Society annual meeting, July 31 – August 4, Anaheim, CA, USA.
- Fraaije BA (2005) 'Application of PCR-based diagnostics to study the evolution of fungicide resistance in populations of *Mycosphaerella graminicola*' The Biennial Nusbaum Conference 'Linking genomic advances to the understanding and management of plant disease', February 27-28, NC State University, Raleigh, USA
- Lucas JA (2005) 'QoI resistance in cereal pathogens: the European experience. Invited speaker at APS Annual Meeting, July 31- August 4, Austin, Texas, USA

Posters

- Lucas JA *et al*. (2003) 'Providing a scientific basis for the avoidance of fungicide resistance in plant pathogens' Sustainable Arable Link Conference, Rothamsted, June 26 (on CD).
- Fraaije BA, Burnett F, Clark WS & Lucas JA (2005) 'Evolution of QoI resistance in populations of Mycosphaerella graminicola' APS Annual Meeting, July 31-August 4, Austin, Texas, USA (on CD)

Knowledge transfer activities

Rothamsted Research

- Clark WS & Fraaije BA (2003) HGCA Topic Sheet No. 69 spring 2003 'Strobilurin resistance in Septoria tritici in the UK (on CD).
- Lucas JA (2003) ARIA/HGCA workshop 'Fungicide resistance diagnostics and their application in resistance management' Newbury.
- Fraaije BA (2003) contribution to Crop Protection magazine-April 2003. Resistance present in UK at start of season
- Lucas JA (2003) Fungicide resistance diagnostics. Workshop session at ARIA Science day Rothamsted, June 20.
- Fraaije BA (2003) contribution to Boerderij/Akkerbouw magazine-December 2003. Septoria resistance to strobilurins
- Lucas JA HGCA/TAG agronomy field days 'Managing resistance' Cirencester (2 June) and Louth (9 June).
- Lucas JA (2003) Cereals. Presentation 'Septoria resistance – strobilurin fungicides' Royston, June.
- Lucas JA (2004) Cereals HGCA poster and field plot demonstration, June 15-16.
- Lucas JA (2004) Crops Magazine and Syngenta Spring solutions live – expert panel. Shuttleworth Agricultural College (19 Feb) and Royal Agriculural College Cirencester (24 Feb).
- Lucas JA (2004) Bayer Crop Science workshop in Huntingdon 'Managing Septoria triazole resistance'
- Lucas JA (2004) HGCA roadshows 'Fungus vs. fungicide – the battle for control' Scotch Corner (7 Jan), Lincoln (20 Jan), Oxford (22 Jan) and Taunton (29 Jan).
- Project Workshop at Rothamsted Research, 14 January. Project consortium members and invited scientists James Brown, Chris Gilligan, Frank van den Bosch, Derek Hollomon and Mike Shaw. Results of workshop included in press release of 21 February (on CD)
- Lucas JA (2005) contribution to Crops Magazine - April 9. Focus back on triazoles.
- Lucas JA (2005) 'Fungicide resistance: strobilurins v. triazoles' Association of Independent Crop Consultants Science in Agronomy Training Day, Rothamsted, September 20.
- Lucas JA (2006) 'Designing a fungicide programme' Association of Independent Crop Consultants Winter Conference. Daventry, January.

- Lucas JA (2006) Avoiding and managing fungicide resistance. Presentation at HGCA Conference, Arable Crop Protection in the balance: Profit and the environment. Grantham, January 25-26.

SAC KT activities

- Burnett FJ Regular articles in SAC Crop Protection Report (20 issues) per annum (800 subscribers) in 2003, 2004, 2005.
- Burnett FJ Regular articles in SAC Agronomy Bulletin (12 issues per annum) 2005
- Burnett FJ (2003) Strategies for managing QoI resistance HGCA monitoring meeting 22 Jan 2003
- Burnett FJ (2003) Winter wheat fungicide programmes, SAC Cereal Group meeting, Stair Arms, Pathhead (5 Feb 2003)
- Burnett FJ (2003) Fungicide Resistance issues. Lecture to BASIS course candidates (12 February 2003)
- Burnett FJ (2003) Fungicide use and decision support systems. Lecture to SAC BTG Applied Crop Technology Course students
- Burnett FJ (2003) Report to Fungicide Resistance Action Group. Huxton 26 March
- Burnett FJ (2003) Fungicide programmes for 2003. Midlothian Cereal Group 23 March 2003.
- Burnett FJ (2003) Fungicide resistance management. Tutorial with Agriculture II students 2 June 03
- Burnett FJ (2003) Fungicide programmes for 2003. West Lothian Cereal Group 23 March 2003.
- Burnett FJ (2003) Trial demonstration and talk. SAC HGCA Banhkhead open Day, Perth 24 June 2003
- Burnett FJ (2003) Trial demonstration and talk. SAC HGCA Duns open evening, Borders 1 July 2003
- Burnett FJ (2003) Fungicide programmes and practical strategies for resistance management. HGCA Topic breakfast Belfast Northern Ireland 19 November 2003
- Burnett FJ (2003) Fungus v fungicide – the battle for control. HGCA Roadshow Maidstone, Kent
- Burnett FJ (2003) Fungus v fungicide – the battle for control HGCA Roadshow Newmarket 18 Dec 2003
- Burnett FJ (2004) Early season disease control. Article in Scottish Farmer 5 January 2004
- Burnett FJ (2004) Fungicide programmes 2004. SAC Advisory Newsletter article 7 Jan 2004
- Burnett FJ (2004) Fungus v fungicide – the battle for control. HGCA Roadshow Crieff, 15 January 2004
- Burnett FJ (2004) Panel member Crops Magazine and Syngenta Spring Solutions Live – expert panel. Royal Highland Show Ground, Ingliston 4 March 2004
- Burnett FJ (2004) Crop Protection issues in winter wheat SAC Technology Transfer Day Perth 16 March 2004
- Burnett FJ (2004) Contribution to Scottish Farmer article on strobilurin resistance 30 April 2004
- Burnett FJ (2004) Trial Demonstration and talk SAC HGCA Perth Open day 29 June 2004
- Burnett FJ (2004) Trial Demonstration and talk SAC HGCA Borders Open day 1 July 2005
- Burnett FJ (2004) Trial Demonstration and talk Scottish Agronomy HGCA Fife Open day 13 July 2004
- Burnett FJ (2004) SAC Technology Transfer day, Atholl Palace Hotel, Pitlochry 8 September 2004
- Burnett FJ (2004) Principals of disease control. Lecture University of Edinburgh, Crop Science students 11 November 2004

- Burnett FJ (2004) Crop protection issues in winter wheat. Cereal Farmers Midlothian Group Meeting , Bush Estate 14 Dec 2004
- Burnett FJ (2004) Crop protection issues in winter wheat. Cereal Farmers West Lothian Group Meeting , Bush Estate 14 Dec 2004
- Burnett FJ (2005) Contribution to article on resistance in Farm Business Management 15 Feb 2005
- Burnett FJ (2005) Resistance management Lecture to BASIS course candidates 24 Feb 2005
- Burnett FJ (2005) Minimising fungicide costs HGCA Topic breakfast Longframlington 3 March 2005
- Burnett FJ (2005) Fungicide programmes for cereals. Assoctaion of Arable Crop Consultants Meeting Stonehaven 12 April 2005
- Burnett FJ (2005) Fungicide programmes for 2005 Cereal Farmers Midlothian Group 13 April
- Burnett FJ (2005) Fungicide programmes for 2005 Cereal Farmers West Lothian Group 13 April
- Burnett FJ (2005) Trial Demonstration and talk SAC HGCA Duns Trial site open evening 30 June 2005
- Burnett FJ (2005) Trial Demonstration and talk SAC HGCA Inverurie Trial site day evening 7 July 2005
- Burnett FJ (2005) Crop Protection issue in winter wheat SAC knowledge transfer day Pitlochry 6 September 2005
- Burnett FJ (2005) Fungicide resistance management Lecture to BASIS course candidates 22 November 2005
- Burnett FJ (2005) Fungicide programmes for 2006. Farmers meeting Station Hotel, Stonehaven 7 December 2005
- Burnett FJ (2005) Fungicide programmes for 2006 Cereal Farmers West Lothian Group 13 December 2005
- Burnett FJ (2006) Early season disease control in winter wheat. Article in Scottish Farmer 24 February 2006

APPENDICES

- Appendix A. Press release from project

Appendices B – E may be purchased from HGCA in CD format by request.

- Appendix B: Raw data of core field trials on barley powdery mildew and Septoria leaf blotch (on CD)
- Appendix C: Minutes of Management Committee (Consortium) meetings (on CD)
- Appendix D: FRAC QoI Working Group Minutes (on CD)
- Appendix E: Syngenta presentation slides on QoI resistance (on CD)

Appendix A: Press release (February 2005)

The development of Septoria resistance to strobilurins was too rapid for effective resistance management.

Septoria and mildew resistance to strobilurin fungicides is now too widespread for effective resistance management, according to results from a Defra sponsored LINK research project*. The four year project found that Septoria resistance developed much faster than anticipated. Resistance was found in around 80% of the Septoria samples tested in 2004, compared to 39% at the start of 2003.

However, strategies to combat resistance are still vital to protect the useful activity of alternative fungicides, particularly as there is evidence that some Septoria isolates are becoming less sensitive to triazoles. To lower the risk of resistance reducing the effectiveness of triazoles, growers should follow current resistance management advice at: www.pesticides.gov.uk\rags_home.asp. Whilst the strobilurins have largely lost their curative effect, there is evidence that they may still provide some useful protectant activity and the research team plans to investigate this further in the coming year.

Take-home messages from the project

- Strobilurins now offer little or no control of mildew in wheat or barley.
- Despite high levels of resistance in the UK, strobilurins can still add to control of *Septoria tritici*, particularly when applied in a protectant mode.
- Strobilurins should always be applied in mixture with an azole fungicide
- The use of fungicide mixtures, where partner fungicides have different modes of action, can help to improve disease control and to slow resistance development.
- Whenever possible use wheat varieties with some resistance to Septoria as this will reduce reliance on fungicides and help to lower the risk of fungicide resistance.

*Sustainable Arable LINK programme "Providing a scientific basis for the avoidance of fungicide resistance in plant pathogens". The LINK consortium comprises Rothamsted Research, ADAS, Scottish Agricultural College, HGCA, BASF, Bayer CropScience, Du Pont and Syngenta.

The Sustainable Arable LINK Programme facilitates government financial support of consortia of industry and academia collaborating to deliver high quality pre-commercial research for their members which will further the sustainable development of the arable sector (http://defrafarmingandfoodscience.csl.gov.uk).

Key findings from the project:

- Strobilurin resistance in barley powdery mildew populations, measured as the frequency of the G143A mutation, increased in untreated plots from 5-10 % in 2002, to 15-20 % in 2003. Stepwise selection was seen after fungicide treatments but this was slower than the selection seen with Septoria.

- Higher dose rates and number of applications increased selection for barley mildew resistance, whereas mixing the strobilurin with a morpholine slowed down resistance development.

- The G143A mutation was first detected in Septoria isolates in the field in 2002 although, using a PCR assay, the mutation was found at low levels in strobilurin-treated leaf samples saved from 2001.

- Extensive monitoring revealed a variable distribution of resistance in Septoria populations sampled throughout the UK in spring 2003, from 14 to 87 %, with an average of 39 %. In 2004, less variation was detected and the average resistance frequency was around 80 %.

- Selection for the G143A mutation in Septoria after strobilurin applications was very rapid, even with only a single low dose. Differences in resistance frequencies measured after treatment with a range of doses, mixtures or alternations were small.

- During 2004, a season with high disease pressure, strobilurins applied on their own (as an experimental treatment) provided some degree of disease control in crops infected by a predominantly resistant Septoria population. Better results were obtained using a programme of triazole applications. However, the best yield responses and disease control were obtained with mixtures of strobilurins and triazoles.

- Glasshouse studies showed that resistant isolates of Septoria with the G143A mutation were partially controlled at high doses when strobilurins were applied preventatively. No control was achieved using curative applications. Hence best yield and disease control are likely to be achieved by protectant applications

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