Resistance of *Zymoseptoria tritici* to azoxystrobin and epoxiconazole in the lower North Island of New Zealand

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Abstract In response to a suspected fungicide resistance problem in the spring of 2013, 23 isolates of *Zymoseptoria tritici* (also known as *Mycosphaerella graminicola*) were collected from wheat fields showing symptoms of speckled leaf blotch around the lower North Island. EC₅₀ values for the collected isolates and three sensitive isolates against rates of azoxystrobin, epoxiconazole and isopyrazam were determined by growth in microplates as measured by light absorbance. Furthermore, greenhouse inoculations on fungicide-sprayed plants were conducted with selected isolates. Resistance to azoxystrobin appears widespread, as does reduced sensitivity to epoxiconazole. Most growers supplying the samples were using epoxiconazole as their standard cereal fungicide but at lower than label rates. A combination of conducive weather, low fungicide rates, resistance to azoxystrobin and reduced sensitivity to epoxiconazole is likely to have contributed to the disease epidemic.

Keywords speckled leaf blotch of wheat, fungicide resistance, Quinine outside Inhibitor, demethylation Inhibitor, Succinate dehydrogenase Inhibitors, reduced sprays.

INTRODUCTION

Speckled leaf blotch caused by *Zymoseptoria tritici* (Desm.) Quaedyvlieg & Crous 2011 (Quaedyvlieg et al. 2011) (also known as *Mycosphaerella graminicola* and *Septoria tritici*) is a major disease of wheat (*Triticum aestivum*) globally and in New Zealand. The pathogen is of most significance in autumn-sown wheat crops where infection of the flag, second and third leaf can cause significant losses (Shaw & Royle 1993; Eyal 1999; Jørgensen et al. 2014). In New Zealand, well-timed fungicide applications play a large role in managing the disease. However, speckled leaf blotch is not the only disease under consideration when applying fungicides to an autumn-sown wheat crop. Major pathogens like stripe rust (*Puccinia striiformis* f.sp. *tritici*),

leaf rust (*Puccinia triticana*) and powdery mildew (*Blumeria graminis* f.sp. *tritici*) are also considerations. To control all foliar diseases, growers are recommended to monitor the crop and, depending on cultivar susceptibility, disease presence and/or rain or irrigation status, apply fungicides at the following times: GS31–32 (1st–2nd node on main stem); GS39 (flag leaf emergence on main stem); GS59–61 (ear emergence – start of flowering) and GS69–72 (post-flowering). An optional application before these sprays at GS30 (end of tillering/start of stem elongation) is recommended if stripe rust is evident (Foundation for Arable Research 2012). This control regime means two to four fungicide applications are typically made over this period.

There are three dominant chemical groups in commonly used cereal fungicides: the QoI fungicides (also known as the strobilurins), the DMIs (the demethylation inhibitors, also known as the triazole or azole group) and the recently introduced succinate dehydrogenase inhibitors or SDHIs.

Zymoseptoria tritici resistance to all three groups has been noted in laboratory or field studies (Skinner et al. 1998; Fraaije et al. 2005; Cools et al. 2005). Field resistance to QoIs is conferred by a single point mutation in the cytochrome *b* gene, which causes a G143a substitution. This prevents the fungicide from binding to the Qo site restoring the ability of the fungus carry out microbial respiration (Fraaije et al. 2005). QoI-resistant isolates of the pathogen are now widespread in Europe (Torriani et al. 2009). While DMI fungicides, like the QoIs, have a single biochemical target site, the mechanism of resistance to the DMIs is polygenic, consisting of three primary mechanisms that can combine to determine resistance levels. These mechanisms are mutations in the target-encoding CYP51 gene resulting in decreased affinity of the protein for inhibitors, over-expression of the target CYP51 gene and increased efflux caused by the over-expression of genes encoding membrane transporters. Resistance expression and cross-resistance within the DMI group is dependent on which combinations of mechanisms are present in any given population (Cools & Fraaije 2013). Consequently, DMI resistance development has been more gradual than with the QoIs although reports of resistance to commonly used DMI fungicides are increasing (Cools 2008; Cools et al. 2011; Stammler & Semar 2011). Regarding the SDHI group, isolates of *Z. tritici* have been found with the necessary mutations in genes encoding for the mitochondrial succinate dehydrogenase (SDH) enzyme to confer resistance. Although the frequency of these mutations for SDHI resistance appears too low to affect field performance, there is a fear that incremental mutations may lead to cross-resistance and eventual loss of control (Sierotzki 2013).

Sensitivity to QoI and DMI fungicides in Canterbury wheat crops were investigated in 2004-2005 and again in 2008 but no resistance was evident (Foundation for Arable Research 2013). However, work during the 2013-2014 seasons showed reduced sensitivities to both of these fungicide groups (R. Craigie, Foundation for Arable Research, personal communication).

Cereals are popular cash crops in the lower North Island. While barley tends to be more favoured, in 2012 there was approximately 2313 hectares of wheat grown producing a total of 14278 tonnes (Statistics New Zealand 2012), which represents 2.9% of the national wheat crop. The region has seen a shift in the last 10 years from spring-sown wheat to autumn-sown wheat. It is estimated that 70% of the lower North Island wheat crop is now planted in the autumn.

In the spring of 2013, speckled leaf blotch of wheat was a serious problem in a number of fields in Manawatu/Rangitikei. One factor may have been the development of resistance to the fungicide groups mentioned above. To investigate this, a number of isolates from several areas in the lower North Island were collected and their resistance status determined by a microplate assay. Fungicide pot trials on selected isolates were also carried out.

MATERIALS AND METHODS

Sample collection

Leaves from wheat plants showing symptoms of speckled leaf blotch were collected from autumn-sown wheat fields in the lower North Island between 16 October and 5 December 2013. The growers were all clients of PGG Wrightson Grain, which services 60-70% of wheat producers in the region. At the time of collection, the fungicides applied to the crop to date were recorded.

Not all the leaf samples received led to successful *Z. tritici* isolations. Some samples had remained moist for too long and had been overrun by saprophytes while others were showing symptoms but had few pycnidia. Of the 29 samples submitted, isolates were successfully recovered from 23.

Isolates labelled 001 to 012, 018, 021, 023 and 023 were from the Manawatu/Rangitikei area; 013 to 017, 022 and 025 were from fields around the Ruapehu area; 026 to 029 were from Wairarapa and 019 to 020 from Central Hawke's Bay.

Along with isolates collected from lower North Island fields, three isolates with known sensitivity to QoI and DMI fungicides were provided by Plant & Food Research.

Isolation

To obtain isolates from plant tissue, a cirrus originating from a single pycnidium from a leaf sample held in high humidity for 24 h was transferred to a drop of sterile water then streaked onto yeast malt agar (YMA) plates. This was then incubated in the dark at 15°C. After colonies had formed, presumably originating from a single spore, a single colony was chosen and streaked onto YMA slants.

Fungicides

Three common cereal fungicides representing the main fungicide groups were included in the assays. These were the QoI azoxystrobin (Amistar® SC), the DMI epoxiconazole (Opus®) and the SDHI isopyrazam (Seguris Flexi®). Ideally technical material is best for the assays but this could not be obtained in the time available. Consequently, the tests were conducted with commercial formulations, with the exception of azoxystrobin which was GLP grade formulation. The use of the formulated product appears routine in commercial testing elsewhere in the world and has found to give similar results to technical grade (Gerd Stammer, BSAF, Germany, personal communication).

Assay

An assay was used to determine the EC₅₀ of each isolate for each fungicide. The EC₅₀ is the concentration at which 50% of the *in vitro* fungal growth is inhibited. The assay followed the method of Siah et al. (2010) for QoI testing of *Z. tritici*, with some modifications. It was similar to assay methods published by the Fungicide Resistance Action Committee (FRAC) for all three fungicide groups. Assays were conducted

using 96-well flat bottomed microplates. Each well contained 150 µl of concentrated liquid glucose peptone growth media amended with fungicides at varying concentrations. Into each well was added a 50 µl spore suspension giving a final concentration of 2 x 10⁵ spores/litre. The media ingredients in the final 200 µl were 14.3 g/litre dextrose, 7.1 g/litre bacto-peptone and 1.4 g/litre yeast extract.

Fungicide concentrations tested were as follows: azoxystrobin (0.001, 0.01, 0.1, 1, 10 and 100 mg/litre), epoxiconazole (0.003, 0.01, 0.03, 0.1, 0.3, 1 and 3 mg/litre), isopyrazam (0.003, 0.01, 0.03, 0.1, 0.3, 1 and 3 mg/litre). The azoxystrobin treatments were amended with 100 mg/litre of salicylhydroxamic acid (SHAM) to prevent false positives through the possible utilisation of an alternative respiration system. Isolates capable of this may indicate resistance *in vitro* but are unlikely to express field resistance (Ziogas et al. 1997; Wood & Hollomon 2003).

Wells including spores but no fungicide were included as controls and wells with media alone were also included in the assay to give a base value for the absorbance. Each isolate was grown in three plates, one plate for each fungicide. Each treatment (i.e. fungicide concentration) was replicated nine times within a plate while media-alone wells were replicated three times for each treatment.

After preparation the microplates were covered with sterile lids, wrapped in tinfoil and incubated at 18°C for 7 days on a shaker at 140 rpm. After this period they were read in a microplate reader at an absorbance of 405 nm.

EC₅₀ values were determined by non-linear regression (dose response curve) using least squares as the fitting method. The software packaged use was GraphPad Prism 6 (Hearne Scientific Software).

Pot trials

In pot trials, full, 75%, 50% and 25% of recommended field rates of azoxystrobin and epoxiconazole product were assessed for their ability to prevent infection and pathogen development against two isolates of *Z. tritici* tested above. These rates equated to 187.5, 140.6,

93.8 and 46.9 g/ha azoxystrobin and 125, 93.8, 62.5 and 31.3 g/ha epoxiconazole. Isolate 007 was chosen as it showed resistance to azoxystrobin and showed a higher EC_{50} than most others to epoxiconazole. Isolate PF 23C2 was sensitive to both fungicides and acted as a baseline.

Wheat plants (cv. Morph) were sown in 10×10 cm planter bags, one seed per bag and grown in a glasshouse until tillering was well underway. Whole plants were then sprayed with the fungicide treatments (4 plants per treatment) using a handheld pressure sprayer calibrated to deliver 2000 litres/ha. After spraying, the plants were left to dry for 4 h while the isolate suspensions were prepared.

The isolates were grown on YMA for 7 days. The plates were then flooded with reverse-osmosis purified water, then filtered through two layers of cheesecloth into a measuring cylinder. The spore concentration was measured with a haemocytometer and adjusted to 5×10^6 spores/ml. Plants were inoculated by spraying as for the fungicides. One drop of Tween was added to the suspension to aid coverage.

Inoculated plants were randomly arranged on benches in a glasshouse. The benches containing the plants were covered with frame draped with transparent cloth and covered in plastic. For 3 days an automatic watering system applied a mist in the evening ensuring the plants were completely wet during the night. Watering from that point on was by capillary matting. After 7 days the frame was removed leaving the plants to grow in normal glasshouse conditions. The glasshouse was not climate controlled and temperatures fluctuated between 23°C in the middle of the day and 12°C at night during the period of incubation and disease development.

After 28 days the plants, which were then just commencing stem elongation, were assessed. The three largest tillers in each pot were selected and the 3rd leaf from the top (two down from the emerging leaf) was scored for the presence of lesions with pycnidia giving a total of 12 leaves scored per treatment. The 3rd leaf was selected as this leaf would have been present when the plants were sprayed, and was not starting to senesce like some of the lower leaves.

Fisher's exact test was used to compare treatments and the chi-square test for trend used to check for a relationship within the fungicide treatments. As above, the package used for analysis was GraphPad Prism 6.

RESULTS

Fungicide applications and rates

Table 1 shows the fungicides used in the field corresponding to the sample number (which equates to the isolate number) up to the date the samples were collected. It is important to note that the fungicides listed are those that had been used in the crop up to the date of sampling, sometime between 16 October and 5 December. They are not, therefore, reflective of the whole spray programme. It is possible for example, that by the time the last few diseased leaves were collected on the 5 December, other fungicide applications may have occurred on the fields where earlier diseased leaves had been gathered. However, the data do indicate fungicide choice and rates of early spring fungicide applications amongst the growers whose fields were sampled. DMI fungicides such as epoxiconazole featured prominently. QoI fungicides such as azoxystrobin or pyraclostrobin were used occasionally in mixture with a DMI. All fields were sprayed with rates of epoxiconazole ranging from 40% to 75% of the recommended rate for speckled leaf blotch (Anonymous 2013). The QoI applications in mixture were also lower than label rates.

None of the growers had used an SDHI fungicide on the crops in question.

Assays

EC_{50} values of isolates from the survey ranged from >0.31 to >100 mg/litre for azoxystrobin + SHAM, 0.06 to 0.96 mg/litre for epoxiconazole and 0.01 to 0.22 mg/litre for isopyrazam (Table 2). In contrast, the three reference isolates ranged from 0.01 to 0.02 mg/litre for azoxystrobin, 0.03 to 0.04 mg/litre for epoxiconazole and 0.03 to 0.05 mg/litre for isopyrazam.

All isolates exhibited EC_{50} values for azoxystrobin greater than $100\times$ in excess of those for the reference ones, with the exception of isolates 004 and 002, which were

Table 1 Fungicide applications prior the time of sampling in wheat crops visited between 16 October and 5 December 2013. Sample numbers equate to isolates obtained from leaves. The “+” symbol indicates two or more applications of a fungicide. Where only the rate is given on the right hand side of the “+” symbol the fungicide applied was the one on the left. The “/” symbol separating two fungicides indicate they were applied together in a mixture.

Samples	Fungicides and rate of each application in g/ha
001	epoxiconazole, 49.8 + 75
002	epoxiconazole, 50 + 75 + 94 / pyraclostrobin, 100
004	prothioconazole, 100
005, 008	epoxiconazole, 50 + 75
006	epoxiconazole, 50 + 75 + 94 / pyraclostrobin, 100
007	epoxiconazole, 50
009	prothioconazole, 150 + epoxiconazole, 94 / azoxystrobin, 87.5
010-011	epoxiconazole, 50 + 62.5 / pyraclostrobin, 87.5
003,012, 013 ¹ -017,	epoxiconazole, 62.5
021 ¹ , 022, 025	
018 ¹	epoxiconazole, 37.5 + 62.5 + tebuconazole, 125 / azoxystrobin, 100
019 ¹	epoxiconazole, 50 + 94 + 62.5 / azoxystrobin, 75
020 ¹	epoxiconazole, 50 + 62.5 / azoxystrobin, 75
023	epoxiconazole, 62.5 + 50 / azoxystrobin, 250
024 ¹	tebuconazole, 187 / epoxiconazole, 75 + tebuconazole, 187 / epoxiconazole, 75 / azoxystrobin, 125
026-028	prothioconazole, 150 + 100 / fluoxastrobin, 50 + prothioconazole, 150 / azoxystrobin, 100

¹Unable to isolate *Zymoseptoria tritici* from infected leaves.

approximately 15× and 30× less sensitive than the known sensitive isolates.

On the other hand, the EC₅₀ values for isopyrazam indicated the isolates are still sensitive to that fungicide although isolate 015 had a higher than expected value.

The EC₅₀ values for epoxiconazole were around 10× higher in most isolates compared to the reference strains with the exception of isolates 004, 026 and 028 where the values are much the same. 026 and 028 both came from adjacent wheat fields managed by the same grower near Masterton.

Pot trials

Results of the pot trial can be seen in Figure 1. Of the 12 leaves assessed over the 4 replicates, the two untreated and the 007/azoxystrobin treatments at all rates showed a very high level of disease incidence with either 11/12 or 12/12 leaves infected. In contrast, full and 50% rates of azoxystrobin prevented infection of PF 23C2 entirely.

A lack of effectiveness against isolate 007 was not so marked using epoxiconazole, although even at the full rate, three leaves from the 12 assessed showed lesions with pycnidia. At the 50% rate, this rose to 8/12 leaves. PF 23C2 was controlled completely by the full rate of epoxiconazole but 2/12 leaves showed pycnidia-bearing lesions with the 25% rate. The chi-square test for trend showed a significant ($P < 0.01$) increase in leaves with pycnidia with reducing rates for both fungicide treatments with isolate 007, and for azoxystrobin with isolate PF 23C2.

DISCUSSION

There is no evidence to suggest any reduced sensitivity to isopyrazam in any of the isolates collected in this study. Even though isolate 015 has a relatively high EC₅₀ compared to the others it is more likely to be due to natural variation in the population than resistance factors, as the SDHI group of fungicides have recently been introduced on the market and have not been used extensively yet in the lower North Island.

Figure 1 Number of leaves showing pycnidia of *Z. tritici* from a total of 12 leaves (third leaf down from the emerging first leaf on the three largest tillers on four plants) sprayed with varying rates of azoxystrobin and epoxiconazole. Letters indicate which counts are significantly different from one another ($P < 0.05$) using Fisher's exact test.

For azoxystrobin the situation is very different. Resistance by *Z. tritici* to this fungicide appears widespread in the region. In the pot trial, azoxystrobin applied at the recommended rate showed no ability to prevent infection by a resistant isolate, albeit under extreme infection conditions. Given resistant isolates appear to be common in the lower North Island it is unlikely QoI fungicides alone would be able to control the disease given favourable conditions for the pathogen. This mirrors the situation in Europe, where QoI resistance to *Z. tritici* and *B. graminis* f. sp. *tritici* is common (Lucas 2006). Anecdotal evidence suggests speckled leaf blotch has been increasing in the Manawatu/Rangitikei in recent years which may, in part, be a reflection of QoI resistance. The greater inoculum present may have contributed to the 2013 epidemic. It is of concern that QoI resistance to *Z. tritici*

is widespread in the region not only in terms of loss of disease control but resistance also negates its role as companion fungicide in mixtures for fungicide resistance management.

Epoxiconazole, the last of the three fungicides tested, was a popular fungicide for the growers covered in the present study. In general, the isolates for epoxiconazole had higher EC_{50} values when compared with the reference isolates, but the difference between reference and collected isolates was less for epoxiconazole than those for azoxystrobin. However, given the EC_{50} values of the reference isolates tested against epoxiconazole and the results of the pot trial, the pathogen population appears to be drifting towards epoxiconazole resistance. Stammiller & Semar (2011) found that recommended field rates of epoxiconazole could adequately control *Z. tritici* even when EC_{50} values approach

Table 2 EC₅₀ values (mg/litre) and confidence intervals of collected and reference isolates for three fungicides as calculated from *in vitro* assays.

	azoxystrobin		epoxiconazole		isopyrazam	
	EC ₅₀	95% C.I.	EC ₅₀	95% C.I.	EC ₅₀	95% C.I.
Sample isolates						
001	14.44	8.88–23.46	0.41	0.28–0.60	0.04	0.03–0.05
002	0.60	0.42–0.85	0.33	0.22–0.48	0.01	0.01–0.02
003	5.95	3.38–10.47	0.72	0.52–1.01	0.08	0.06–0.10
004	0.31	0.20–0.64	0.06	0.04–0.07	0.07	0.06–0.08
005	85.54	46.09–158.8	0.18	0.11–0.27	0.06	0.05–0.08
006	2.10	1.21–3.63	0.21	0.15–0.28	0.02	0.02–0.03
007	12.75	5.62–28.95	0.96	0.70–1.32	0.05	0.04–0.06
008	>100		0.60	0.36–1.00	0.05	0.04–0.07
009	3.16	1.74–5.72	0.53	0.33–0.87	0.02	0.02–0.03
010	47.15	23.22–95.74	0.60	0.37–0.98	0.09	0.07–0.11
011	56.18	25.19–125.4	0.44	0.29–0.65	0.03	0.02–0.03
012	4.10	2.15–7.84	0.22	0.13–0.38	0.02	0.02–0.03
014	19.46	7.74–48.91	0.35	0.24–0.53	0.05	0.04–0.06
015	12.58	4.91–32.26	0.21	0.12–0.37	0.22	0.14–0.34
016	4.30	2.34–7.90	0.47	0.33–0.68	0.10	0.08–0.14
017	3.67	2.06–6.56	0.73	0.42–1.26	0.03	0.02–0.03
022	25.84	10.89–61.35	0.29	0.22–0.39	0.02	0.02–0.03
023	3.47	1.93–6.22	0.19	0.15–0.24	0.03	0.02–0.05
025	3.77	1.95–7.27	0.52	0.38–0.71	0.06	0.05–0.08
026	15.22	5.01–46.24	0.07	0.05–0.09	0.08	0.06–0.11
027	32.59	9.81–108.4	0.76	0.47–1.20	0.03	0.03–0.04
028	12.00	7.25–19.87	0.03	0.02–0.05	0.03	0.02–0.04
029	15.66	7.47–32.83	0.54	0.36–0.81	0.04	0.04–0.05
Reference Isolates						
PF 23C2	0.02	0.01–0.05	0.04	0.03–0.05	0.05	0.04–0.06
PF 23C3	0.02	0.01–0.03	0.04	0.03–0.06	0.03	0.03–0.04
PF 23C4	0.02	0.01–0.04	0.04	0.03–0.05	0.04	0.04–0.05

1.0 mg/litre. The pot trial showed some infection at full rates of epoxiconazole with an isolate with an EC₅₀ of approximately 0.96 mg/litre. However, the pot trial does not reflect the field situation fully. Plants and fungicides were challenged with a high inoculum load and ideal infection conditions. Nevertheless this result adds weight to the evidence that resistance to epoxiconazole as measured in the laboratory may translate to infection in the field given a susceptible host and the right environmental conditions.

This research was carried out to determine if fungicide resistance was the primary cause of speckled leaf blotch outbreaks in Manawatu/Rangitikei in the spring of 2013. Certainly, resistance to QoI fungicides and loss of sensitivity to epoxiconazole appears to be present in the

pathogen population. This may have had a role to play but it is unlikely to be the only cause. Another factor could have been the weather. In September of 2013, much of the region surveyed had more than 150% of normal rainfall (NIWA 2013). Crops are likely to have remained wet for over 72 h, which is more than enough for successful infections (Eyal et al. 1987). The rate at which the fungicides were applied may also have contributed. Information collected during the study showed that growers were commonly using lower than the recommended label rates of fungicide. Furthermore it appears to have been a widespread practice for a number of years. Van Toor et al. (2013) found applying lower than recommended rates of cereal fungicides was a common practice in cereal crops in their

2008-2009 survey, with only one-third of DMI fungicides applied at the full rate. Surveyed growers were commonly using epoxiconazole rates between 30 to 60% of the label rate.

The participating growers were all clients of one large merchant firm but anecdotal evidence would suggest the use of low rates is common across the sector, at least in the region under study. The widespread usage of these low rates has come about from industry trials, which show that rates could seemingly be safely lowered while diseases in general were held at bay given good spray timing and the appropriate cultivars (Foundation for Arable Research 2012). In doing so the cost of production is reduced. However, most trials were conducted in Canterbury where speckled leaf blotch has been regarded as less of a problem in autumn-sown crops when compared to the UK and Northern Europe (Foundation for Arable Research 2009). Rust diseases are more likely to be the target of the early sprays at GS30-GS32.

Historically speckled leaf blotch has been less significant in the Manawatu/Rangitikei area than Canterbury. In contrast to North/Mid-Canterbury and Southland, a 1995-1996 survey by Braithwaite et al. (1998) found no incidence of the disease in the Wanganui and Wellington regions in the 16 sites surveyed. At that time virtually the entire wheat crop in those regions is likely to have been spring sown. This is not the case at present. In the past 10 years there has been a move towards autumn-sown wheat crops in the lower North Island. This has meant there is less industry experience or local knowledge of potential diseases for autumn-sown wheat than in areas where autumn sowing is common. Furthermore, the prevalence and importance of diseases in Manawatu/Rangitikei wheat crops are likely to differ from Canterbury. The cool, wet conditions typifying spring on the western side of the southern North Island are conducive for *Z. tritici* infection of autumn-sown crops. Given higher disease pressure is more likely in the lower North Island than in Canterbury, where most disease control trials have been carried out, the use of reduced fungicide rates in the former region may not be tenable.

It should be pointed out that the Foundation for Arable Research (FAR) states that "Use of rates lower than the full label rate is done at the grower's own risk." It also recommends prothioconazole be used in place of epoxiconazole for early sprays where speckled leaf blotch is likely to be a threat (Foundation for Arable Research 2012). However, most of the affected growers in the present study used epoxiconazole as an early spray. While epoxiconazole suppresses *Z. tritici* growth it is less effective than some other fungicides. For example, both Marroni et al. (2006) and Godwin et al. (1994) found epoxiconazole did not inhibit the early stages of a *Z. tritici* infection as much as azoxystrobin (assuming a sensitive strain of the pathogen).

It is important to note that low fungicide rates are not being suggested as the cause of azoxystrobin or epoxiconazole resistance in this study. Indeed, recent research suggests low rates of high-risk fungicides do not accelerate resistance development (van den Bosch et al. 2011; Hobbelen et al. 2014). The problem with low rates is that they can fail to check disease where environmental conditions are conducive for infection and spread of pathogens, especially those with reduced sensitivity to the fungicide.

In conclusion, both azoxystrobin and epoxiconazole resistance may have been a factor in the high levels of speckled leaf blotch in the lower North Island in the spring of 2013 but environmental conditions and a lack of experience with, and knowledge of the significance of, the disease in autumn-sown crops in the region (resulting in low fungicide rates and less than ideal fungicide selection) is also likely to have played a part.

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