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# Fungicide sensitivity and resistance in the blackleg fungus, *Leptosphaeria maculans*, across canola growing regions in Australia

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## ABSTRACT

Fungicide use has become a fundamental part of many crop protection systems around the world, including to control blackleg disease on canola (*Brassica napus* L.). In Australia, most canola growers routinely apply at least one fungicide, and potentially multiple fungicides with different modes of action, in a single growing season. There is evidence for the emergence of fungicide resistance in *Leptosphaeria maculans*, the causal agent of blackleg disease, to the demethylation inhibitor (DMI) class of fungicides in Australia. However, it is not known whether resistance exists towards other chemical classes such as the succinate dehydrogenase inhibitors (SDHI). In this work, 397 samples were screened for resistance towards seven fungicide treatments in stubble-borne *L. maculans* populations collected from eight canola-growing agro-ecological regions of Australia from 2018 to 2020, a time frame that bridges the introduction of new chemicals for blackleg control. We confirmed that DMI resistance in *L. maculans* is pervasive across all of the sampled canola-growing regions, with 15% of fungal populations displaying high levels (resistance scores >0.5) of resistance towards the DMI fungicides. Although resistance to newly introduced SDHI fungicides was low, we found evidence of positive cross-resistance between established DMI-only fungicides and a newly introduced combined DMI and quinone outside inhibitor fungicide, suggesting that the efficacy of the latter may be limited by widespread DMI resistance. Proactive surveillance, as performed here, may provide a means to avoid the rapid loss of fungicide efficacy in the field.

**Keywords:** blackleg disease, DMI fungicides, fungicide resistance, fungicide sensitivity, *in planta* assays, population surveys, $Q_O$I fungicides, SDHI fungicides.

## Introduction

Fungal plant pathogens can be a major cause of yield loss in all cropping systems worldwide. The use of fungicides to minimise the impact of these pathogens has become an integral part of most disease management strategies, and blackleg disease of canola (*Brassica napus* L.) is no exception (Fitt *et al.* 2006). The pathogen *Leptosphaeria maculans* causes blackleg disease, which is one of the major fungal diseases of canola (Fitt *et al.* 2006; Zheng *et al.* 2020). With the exception of China, this fungus has been reported in all canola-growing regions and results in yield losses of 5–50% in Europe, Canada and Australia, with localised epidemics resulting in up to 90% yield loss (Fitt *et al.* 2006; Sprague *et al.* 2006; Zheng *et al.* 2020). Blackleg is a stubble-borne disease whereby the sexual ascospores are released from the crop debris (stubble) during each rainfall event once the spores are mature (Hammond *et al.* 1985). Ascospores land on the leaves of seedlings and grow down the petiole into the stem where the fungus then colonises the vascular tissue, causing crown cankers towards the end of the growing season (Hammond *et al.* 1985). More recently, in Australia, flowers, upper stems and branches can also be infected, leading to upper canopy infection (Sprague *et al.* 2018). Management of blackleg disease involves a

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combination of genetic resistance, cultural practices and fungicides; however, at least in Australia, the use of fungicides has become a major strategy for minimising disease (Van de Wouw *et al.* 2016, 2021).

Fungicide use in canola crops has increased in Australia over the past 20 years; a survey by Van de Wouw *et al.* (2021) found that 95% of canola growers currently use fungicides, versus only 52% in the 2000. This shift towards almost universal use of fungicides has been driven by changes such as increased area sown to canola and tighter rotations, which limit the ability for growers to employ cultural practices that can minimise blackleg disease, such as sowing into paddocks that are isolated from previous years' stubble (Van de Wouw *et al.* 2021).

Three classes of fungicides are registered in Australia for managing blackleg: the demethylation inhibitors (DMIs), the succinate dehydrogenase inhibitors (SDHIs), and the quinone outside inhibitors ($Q_O$Is) (Australian Pesticides and Veterinary Medicines Authority, www.apvma.gov.au). DMIs, also known as azole fungicides, target the cytochrome P450 enzyme 14α-demethylase, encoded by the *ERG11/CYP51* gene (Joseph-Horne and Hollomon 1997). Four fungicides of this class are registered for blackleg control in Australia. Fluquinconazole (registered name Jockey® Stayer) is a seed dressing, and flutriafol (e.g. registered name Impact® In-Furrow) is a dressing applied to fertiliser. These two products have been commercially available for blackleg control in Australia since 2003 and 1997, respectively, and are now used in combination on 55% of crops, and as single treatments on a further 22% (fluquinconazole only) and 18% (flutriafol only) of crops (Van de Wouw *et al.* 2021). The third DMI fungicide for blackleg control in Australia is a mixture of two actives, tebuconazole and prothioconazole (registered name Prosaro®). This fungicide, registered in 2011, was the first foliar fungicide available for blackleg control. In May 2021, a fourth fungicide (registered name Proviso®) was registered for blackleg control; it is a foliar fungicide with prothioconazole as a single active.

The second class of fungicides available for blackleg control, the SDHIs, target the mitochondrial SDH enzyme, interfering with mitochondrial respiration (Sierotzki and Scalliet 2013). Four SDHI fungicides have recently been registered for blackleg control in Australia – two as seed treatments and two as foliar treatments. Pydiflumetofen is the active ingredient in both a seed dressing (registered name Saltro®) and a foliar spray (registered name Miravis®), released in 2020 and 2019, respectively. Fluopyram (registered name ILeVO®) was released in 2020 as a seed dressing. Bixafen, in combination with the DMI prothioconazole and registered under the name Aviator® XPro, is a foliar fungicide released in 2016.

The third class of fungicides registered for blackleg, the $Q_O$Is, also known as strobilurins, act by inhibiting respiration by binding to the $Q_O$ site of cytochrome b, part of the cytochrome bc1 complex (Bartlett *et al.* 2002). Two $Q_O$I-containing fungicides have been registered for blackleg control in 2021: Veritas® Opti, which is a mixture of the strobilurin azoxystrobin and the DMI tebuconazole; and Maxentis® EC, which is a mixture of azoxystrobin and the DMI prothioconazole. Although both of these fungicides have only recently been registered for blackleg control in Australia, Veritas® (with the same actives as Veritas® Opti) has been registered for control of Sclerotinia stem rot in canola since 2016.

Seed dressings and fertiliser-amended fungicides have been available for two decades and are now widely used in Australia. These fungicide options are applied before or during sowing and are aimed at controlling crown canker through the protection of the seedlings. Foliar fungicides have only been available since 2011 and have provided growers with an in-season option to minimise disease. These foliar fungicides can be applied at either the 4–10-leaf stage, with the intention of controlling crown canker through the protection of seedlings, or at 30% bloom, with the intention of controlling upper canopy infection. Despite the relatively recent introduction of foliar fungicides, 49% of Australian growers consider applying them when seasonal conditions are favourable for disease, and a further 20% of Australian growers always apply a foliar fungicide (Van de Wouw *et al.* 2021).

The heavy reliance on fungicides to control fungal pathogens can lead to selection of resistance toward moderate- and high-risk fungicide modes of action (Hollomon 2015). Until 2016, all fungicides registered in Australia belonged to the same fungicide class, the DMIs, likely resulting in strong selection for DMI resistance. Indeed, in a small-scale survey in 2015, 15% of *L. maculans* populations were identified as containing isolates with resistance to fluquinconazole (Van de Wouw *et al.* 2017).

Research on fungicide efficacy tends to be a focus only after resistance has emerged. Consequently, there is often limited knowledge on resistance status prior to fungicide use, the time taken for the resistance to occur initially and then increase in frequency in the population, and how the resistance occurred. However, with the recent introduction of SDHI fungicides in Australia, there is a unique opportunity to look at the baseline resistance present in populations and monitor for changes as the use of these fungicides increases. Early detection of fungicide-resistant populations may be key to managing these chemistries successfully and maintaining their effectiveness over the long term.

In the present study, we surveyed 397 *L. maculans* populations for the presence of resistance to all fungicides registered for blackleg control at the time of the surveys, as well as to a $Q_O$I fungicide used for Sclerotinia stem rot control. The results provide the current status of resistance across all the major canola growing regions in Australia, providing a benchmark for these newly introduced fungicides and evidence of increasing levels of resistance to the DMI class.

## Materials and methods

### Stubble sampling and preparation

In total, 397 canola stubble samples, each representing an individual *L. maculans* population, were screened across the 3 years of the survey (Fig. 1; Supplementary Table S1, Fig. S1). Growers and/or agronomists collected and submitted stubble samples each year from their or their clients' fields, comprising 358 of the 397 stubble samples. A further 39 stubble samples were collected from research field sites from across Australia. For each sample submission, 20 pieces of stubble ~30 cm in length, including the crown of the plant, were collected post-harvest (between November and March) and sent to Horsham, Victoria. Stubble was matured naturally on bare earth from April through to July to allow *L. maculans* growing as a saprotroph in the material to undergo sexual reproduction and for the pseudothecia (sexual fruiting bodies) to mature into producing ascospores. Stubble was placed in mesh bags, allowing stubble samples to be kept separated. Once mature (in July), stubble was used in the *in planta* assays as described below.

With each stubble sample, specific information was supplied from the person submitting the sample, including the location of the crop (either GPS details or closest town), the canola variety sown, and details of all fungicides applied to the crop during the growing season. Similar information was collected from the 2015 fungicide resistance survey published in Van de Wouw *et al.* (2017) and has been used in this study.

### *In planta* assays for detecting fungicide resistance

The ascospore shower technique (Van de Wouw *et al.* 2017; Yang *et al.* 2020) was adapted to screen for resistance to all fungicides currently commercially available for controlling blackleg disease. Canola cv. ATR-Stingray, harbouring the resistance gene *Rlm3*, was used in all *in planta* assays because this resistance gene has been rendered ineffective in Australian populations, and therefore all isolates were expected to be virulent towards this cultivar (Van de Wouw *et al.* 2018). Seven different fungicides were screened in the *in planta* assays and were applied as either a seed-dressing or a foliar application as per label rates (Table 1). With the exception of Veritas®, all fungicides used in this study are registered for blackleg control in Australia. Veritas® was included in this study because it is registered and used widely for control of Sclerotinia stem rot, another fungal disease of canola, and applications of this fungicide may inadvertently be controlling blackleg disease, potentially leading to selection of fungicide resistance within *L. maculans* populations. Since the screening of these stubble populations, a product with the same actives as Veritas® has been registered for blackleg control in Australia under the name Veritas® Opti. The fungicides Maxentis® EC (azoxystrobin + prothioconazole) and Proviso® (prothioconazole) were not screened in this survey because they were only registered for use on canola crops in 2021. Flutriafol is the active ingredient from the fertiliser-applied fungicide Impact® n-Furrow. Owing to practical limitations of using a fungicide combined with fertiliser in the *in planta* assay, flutriafol was instead applied as a foliar fungicide. Given that this fungicide was not being used as per the fungicide label, we refer to this chemical as flutriafol, rather than its commercial product name, throughout the manuscript.

Seed-dressing fungicides were applied to 5 g seed before sowing, using a pipette and gentle agitation of the seed to ensure even coverage of the fungicide. Foliar fungicides were applied to 10-day-old seedlings, 2 days before inoculation, using a hand-held spray unit. The spray unit was calibrated for flow rate (1.67 L/min) and boom width (1.47 m), which were used to calculate the speed of application required for each active ingredient; for example, to obtain a

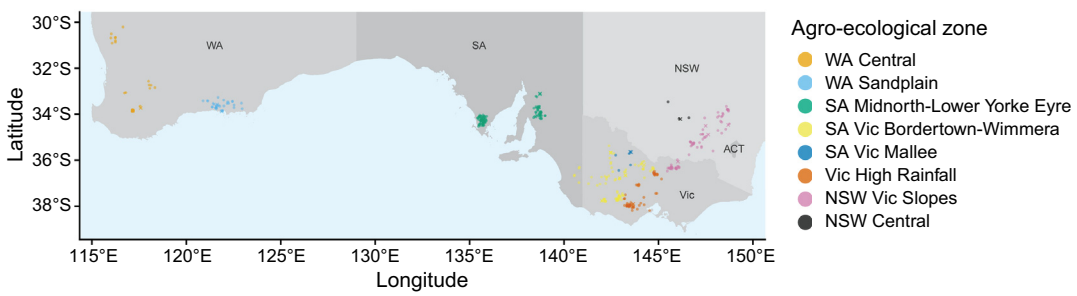


**Fig. 1.** Map of canola stubble sample locations of origin within Australia, with sample icons coloured by their position within, or just adjacent to, a Grains Research and Development Corporation (GRDC) agro-ecological zone. If exact sample coordinates are not known, the position of the point has been randomly moved 0–0.04 units away from the centre of the closest town, to avoid overplotting. Samples that produced lesions on ≤20% of the untreated seedlings in the *in planta* fungicide screen (and were excluded from resistance score calculations) are represented with a cross; samples that produced lesions on >20% of untreated plants are represented with a filled circle. For the same data split by year, see Fig. S1. WA, Western Australia; SA, South Australia; NSW, New South Wales; Vic, Victoria; ACT, Australian Capital Territory.

**Table 1.** Details associated with the fungicides used in this research.

| Fungicide(s) | Commercial product | Fungicide class (FRAC classification) | Timing of application | Application rate | Active constituents | Year of release |
|---|---|---|---|---|---|---|
| Fluquinconazole | Jockey® Stayer | DMI (3) | Seed dressing | 20 L/t | 167 g/L | 2003 |
| Pydiflumetofen | Saltro® | SDHI (7) | Seed dressing | 2 L/t | 200 g/L | 2020 |
| Fluopyram | ILeVO® | SDHI (7) | Seed dressing | 8 L/t | 380 g/L | 2020 |
| Flutriafol | Impact® In-Furrow | DMI (3) | Foliar | 400 mL/ha | 250 g/L | 1997 |
| Tebuconazole + prothioconazole | Prosaro® | DMI (3) | Foliar | 450 mL/ha | 210 g/L + 210 g/L | 2011 |
| Bixafen + prothioconazole | Aviator® XPro | SDHI (7) + DMI (3) | Foliar | 550 mL/ha | 75 g/L + 150 g/L | 2016 |
| Azoxystrobin + tebuconazole | Veritas® | $Q_oI$ (11) + DMI (3) | Foliar | 1000 mL/ha | 120 g/L + 200 g/L | 2016 (SSR), 2021 (blackleg) |

FRAC, Fungicide Resistance Action Committee; DMI, demethylation inhibitors; SDHI, succinate dehydrogenase inhibitors; $Q_oI$, quinone outside inhibitors (strobilurin fungicides). Year of product registration based on Australian Pesticides and Veterinary Medicines Authority (https://apvma.gov.au/); SSR, sclerotinia stem rot registration.

coverage of 1 L/100 $m^2$, an application speed of 35.84 s would be required. This equates to an application speed of 2.6 s along a 5-m plot. Seedlings were placed on the ground and fungicides were applied using the spray unit at a speed of application (2.6 s/5 m) equivalent to the on-farm rate for each active, as described in Table 1.

Seedlings were grown in a controlled environment glasshouse (22°C). Seed was sown at a depth of 1 cm into Scotts Osmocote Premium Plus Superior Potting Mix in 4-cell punnets. The seven fungicide treatments and an untreated control were screened for each stubble sample (blackleg population), using three replicate punnets of canola seedlings, with each of these replicated punnets containing either four plants (2019 and 2020 screens) or eight plants (2018 screens). Therefore, the 2019 and 2020 screens had 12 plants (3 punnets × 4 plants) for each treatment, whereas the 2018 screens had 24 plants (3 punnets × 8 plants) for each treatment (Fig. 2). The replicated punnets were fully randomised within the inoculation boxes prior to inoculation.

The 20 stubble pieces from each sample were moistened and then suspended above the seedlings. The inoculation boxes were then sealed and left for 36 h at 100% humidity to allow ascospores to fall and infect the underlying seedlings (Fig. 2*a–c*). Following inoculation, plants were removed from the inoculation boxes and placed in the glasshouse (22°C) to allow lesion development.


**Fig. 2.** Methodology associated with the *in planta* assays used for fungicide resistance screening in *L. maculans* populations. (*a*) Each treatment had three replicate punnets (each with four or eight plants) that were randomised within the inoculation box: U, untreated; J, Jockey® (DMI); P, Prosaro® (DMI); F, flutriafol (DMI); V, Veritas® ($Q_oI$ + DMI); S, Saltro® (SDHI); A, Aviator® XPro (SDHI + DMI); I, ILeVO® (SDHI). (*b*) Stubble was suspended above the seedlings for 36 h to allow infection. (*c*) Stubble and seedlings were contained within an inoculation box and stubble was moistened regularly. (*d–f*) At 14 days post-inoculation, seedlings were assessed for the presence of lesions (arrows); examples of (*d*) untreated, (e) Jockey-treated, and (*f*) Saltro-treated seedlings.

Lesion development was scored at 14 days post-inoculation (Fig. 2*d–f*). The number of infected cotyledons (determined by the presence of at least one lesion on the cotyledon) was determined for each seedling, and the proportion of cotyledons infected was determined for each replicate punnet for each treatment.

A normalised resistance score (RS) for a particular fungicide treatment was calculated for each stubble sample by dividing the proportion of infected cotyledons on treated seedlings by the proportion of infected cotyledons on untreated seedlings. In two instances where calculated RS values were >1 because of slightly higher disease on treated seedlings, the RS value was set at 1. An RS of 0 indicates that the sample produced no disease on treated plants and the fungal population has no detectable fungicide resistance; an RS of 1 indicates that the sample produced the same amount of disease on treated plants as on untreated plants, and the population has a high frequency of fungicide resistance.

## Data and statistical analyses

Data processing and analyses were performed in R (ver. 3.6.1; R Core Team 2019). Samples were filtered for untreated infection rates of ≤20% by using the 'filter()' function in the *dplyr* package (ver. 0.8.3; Wickham *et al.* 2019). Samples were placed into one of four resistance categories for each fungicide treatment, defined as follows: no resistance, RS = 0; low resistance, RS >0 and ≤0.1; moderate resistance, RS >0.1 and ≤0.5; and high resistance, RS >0.5. Samples were grouped into GRDC agro-ecological zones based on their location of origin (https://grdc.com.au/about/our-industry/growing-regions). Twenty-two samples were outside the formal boundaries of all agro-ecological zones and were re-categorised into their closest zone, from the areas of Benalla and Yass to NSW Vic Slopes, and from Bacchus Marsh West, Colac, Lara and Melb-Nth West to Vic High Rainfall.

Changes in mean RS values for each fungicide treatment across each agro-ecological zone and year were analysed by Kruskal–Wallis rank sum tests, using the 'kruskal.test()' function in the *stats* package (ver. 3.6.1), with *P*-values (Table S2) adjusted for seven multiple comparisons by Bonferroni correction, using a pre-adjustment $\alpha = 0.05$.

Correlation of resistance between pairs of fungicide treatments was estimated by calculating Kendall's tau-b statistic for ordinal contingency tables of resistance category counts, using the 'KendallTauB()' function in the *DescTools* package (ver. 0.99.29; Signorell 2021) to calculate 95% confidence intervals of estimates, and the 'cor.test()' function in the *stats* package to calculate *P*-values. Confidence intervals and *P*-values (Table S2) were adjusted for 21 multiple comparisons by Bonferroni correction, using a conservative pre-adjustment $\alpha = 0.01$.

Fungicide use data were analysed for 358 samples submitted in 2018, 2019 and 2020 by agronomists and growers only; samples collected from field trials were excluded because they were not representative of farming practices. Fungicide use data submitted with stubble samples represent fungicide applications on the previous year's crop, and as such, the data from 2018, 2019 and 2020 samples are referred to as from (the 'use-years' of) 2017, 2018 and 2019, respectively. These data were used to determine changes in fungicide practices across the years surveyed. Data from stubble samples received in 2015 that represent the 2014 fungicide use-year, published previously (Van de Wouw *et al.* 2017), were also analysed for comparison.

## Confirmation of the detection of fungicide resistance using *in planta* assays

In order to confirm that the *in planta* assays were indeed detecting fungicide-resistant isolates, each year isolates were cultured from a subset of lesions that developed on seedlings treated with various fungicides, using methods previously described (Van de Wouw *et al.* 2017). Once re-isolated, individual isolates were inoculated onto cotyledons of seedlings treated with Jockey or Veritas, as previously described, to confirm the reduced sensitivity to fungicides (Van de Wouw *et al.* 2017).

# Results

## Incidence of fungicide resistance across Australia

Of 397 stubble-borne *L. maculans* populations collected from across Australia between 2018 and 2020 and screened for resistance to the DMI, SDHI and $Q_OI$ fungicides, 28 produced an overall cotyledon infection rate ≤20% on untreated seedlings, suggesting that they produced few viable ascospores in the screen; they were excluded from further analyses (Table S1). For the remaining 369 populations, high frequencies of resistance (RS >0.5) were detected for the DMI fungicides; flutriafol and Jockey® (fluquinconazole) had the highest frequency of resistance, with 15.4% and 11.1% of populations with high RS, and 50.9% and 51.5% of populations with moderate RS (>0.1 and ≤0.5), respectively (Figs 3 and 4). Resistance was also detected to the third DMI fungicide, Prosaro® (prothioconazole and tebuconazole); however, it was at a lower frequency, with only 1.4% of populations having high RS and 35.8% moderate RS (Figs 3 and 4).

Conversely, for SDHI fungicides, low or no resistances (RS ≤ 0.1) were detected for 99.2%, 99.1% and 98.9% of populations to Aviator® XPro (bixafen + prothioconazole), Saltro® (pydiflumetofen) and ILeVO® (fluopyram), respectively (Figs 5 and 6); moderate resistances were detected for the remaining populations. For the $Q_OI$-containing fungicide Veritas® (azoxystrobin and tebuconazole), 0.5% of populations scored high RS and 13.8% moderate RS; the remaining populations fell into the low or no resistance categories (Figs 3 and 4).

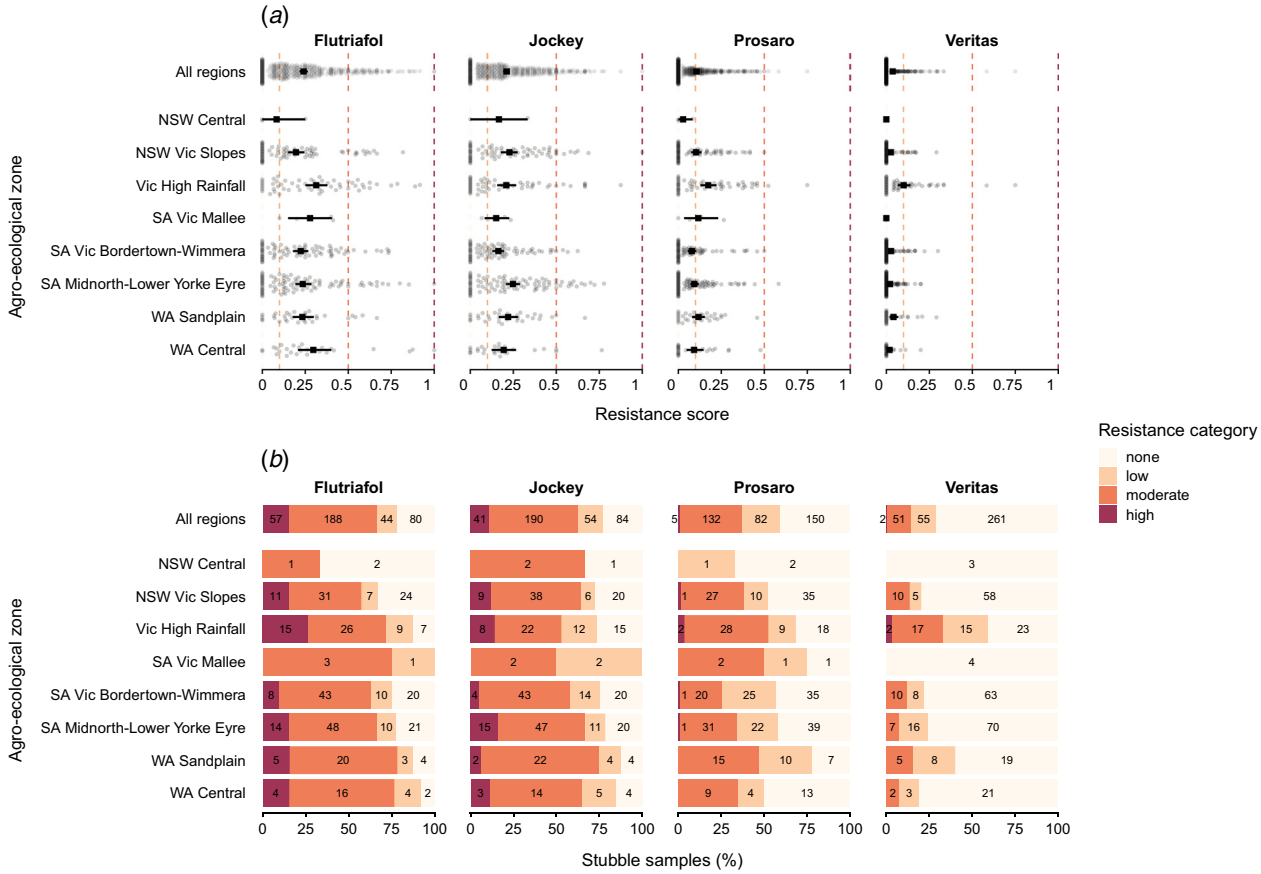


**Fig. 3.** Resistance scores (RS) for 369 stubble-borne *L. maculans* populations to the four DMI-containing fungicide treatments, for each of eight GRDC agro-ecological zones as well as across all sample locations. Flutriafol, Jockey® and Prosaro® are all DMI-only treatments, and Veritas® is a combined DMI + $Q_OI$ treatment. (*a*) Individual RS values for each sample (grey points), with a zone-wide mean (black square) and 95% confidence interval of the mean (black line). Upper RS limits for each of the four resistance categories are marked with a coloured dashed line. (*b*) The proportion of samples per zone falling into one of the four resistance categories: none (RS = 0), low (RS >0 and ≤0.1), moderate (RS >0.1 and ≤0.5), high (RS >0.5). The number of samples within each category (if >0) is indicated by the number in each bar segment.

Each of the samples screened was designated into an agro-ecological zone based on collection location. These zones represent regions with similar climatic parameters, corresponding to comparable levels of agricultural potential, such as canola intensity. For example, zones such as Vic High Rainfall and SA Vic Midnorth–Lower Yorke Eyre are high-production regions and therefore experience high levels of blackleg disease due to increased stubble load and favourable environmental conditions such as rainfall. By contrast, the SA Vic Mallee zone is a low-rainfall and low-production region, and therefore a low blackleg disease region. Significant differences in the mean RS across agro-ecological zones for each treatment were found only for Veritas ($P = 2.07 \times 10^{-6}$; Figs 3 and 5, Table S2). Likewise, significant differences in the mean RS across the year of stubble collection for each treatment were found only for Veritas ($P = 1.97 \times 10^{-9}$; Figs S2 and S3, Table S2).

In order to confirm that the *in planta* assays were indeed detecting isolates with shifts in sensitivity to fungicides, infected leaf tissue was collected to re-isolate cultures. Eight isolates were collected from either the DMI fungicide treatments or $Q_OI$ + DMI treatment and were confirmed to have resistance to DMI fungicides (Table S3). None of the isolates obtained from the $Q_OI$-treated seedlings were confirmed as having shifts in sensitivity to Veritas® but they were resistant to Jockey®, suggesting that populations with high Veritas® RS values contain substantial resistance to the DMI but no resistance to azoxystrobin (Table S3). No isolates were obtained with reduced sensitivity to the SDHI fungicides (data not shown).

## Correlations between chemistries

Correlations of resistance between pairs of fungicide treatments were estimated by calculating Kendall's tau-b statistic (Fig. 7, Fig. S4; for *P*-values, see Table S2). The strongest significant positive correlations (0.256–0.348) were detected for each of the DMI-only fungicide comparisons,

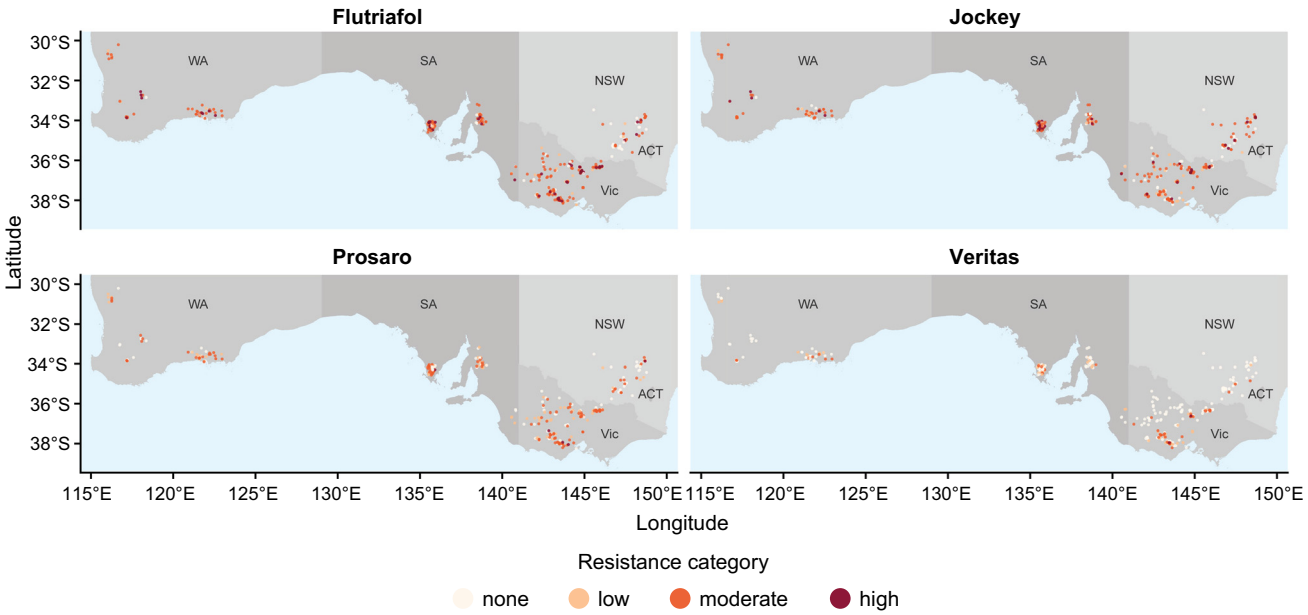


**Fig. 4.** Map of canola stubble sample locations of origin within Australia, with sample icons coloured by their resistance category for each of the four DMI-containing fungicide treatments. If exact sample coordinates are not known, the position of the point has been randomly moved 0–0.04 units away from the centre of the closest town, to avoid overplotting. WA, Western Australia; SA, South Australia; NSW, New South Wales; Vic, Victoria; ACT, Australian Capital Territory. Resistance categories: none (RS = 0), low (RS >0 and ≤0.1), moderate (RS >0.1 and ≤0.5), and 'high' (RS >0.5).

as expected considering their common mode of action. Significant positive correlations were also detected between flutriafol and Veritas® (0.193), Prosaro® and Veritas® (0.236), and between Prosaro® and Saltro® (0.166). Small negative correlations were detected between Aviator® XPro and Saltro® (−0.028) and between Aviator® XPro and ILeVO® (−0.029); these associations were likely due to the high proportion of samples that had no resistance detected. Likewise, the (unexpected) positive correlation between Prosaro® and Saltro® resistance is likely due to the small number of samples ($n$ = 15) with non-zero Saltro® RS values.

### Increases in fungicide use across Australia

Based on the information submitted by agronomists and growers with each stubble sample, seed-dressing fungicide use was high during each year of the survey, with 88–96% of growers applying at least one seed-timing fungicide (Fig. 8). The combined use of Jockey® and flutriafol was the most common seed-dressing practice, with use ranging from 32% to 50% across the survey (Fig. 8*a*).

The use of foliar fungicides has changed across the survey years. Although 4–10-leaf foliar applications have steadily decreased from 7% in 2014 to 0% in 2019, 30% bloom application have rapidly increased from 11% in 2014 to 45% in 2019 (Fig. 8*b*). This is driving an increase in total fungicide applications in a growing season, with a shift from a single fungicide application to two or three applications within a growing season (Fig. 8*c*). With the recent introduction of the SDHI chemistries, there has been a shift towards their use, with 42% of samples having received an Aviator® XPro application and 58% a Prosaro® application at the 30% bloom growth stage in 2019 (Table S1).

## Discussion

Fungicide resistance threatens to overcome the essential role currently played by chemical protection against plant diseases in numerous crops (Fisher *et al.* 2018). For *L. maculans*, fungicides have been used on canola crops for more than two decades in Australia, Canada and the United Kingdom without any field-based reports/observations of resistance developing (Eckert *et al.* 2010; Van de Wouw *et al.* 2016; Zhang and Fernando 2018). However, a 2015 survey showed that resistance to the DMI fungicide fluquinconazole was present in 15% of Australian *L. maculans* populations (Van de Wouw *et al.* 2017). This work has been extended in the present study to include all commercial fungicides registered for blackleg control in Australia. Similar to the findings of Van de Wouw *et al.* (2017), 10–15% of populations displayed high levels of resistance towards the fluquinconazole (Jockey®) and flutriafol fungicides, whereas only 1.4% of populations showed high levels of resistance to the prothioconazole + tebuconazole mixture (Prosaro®). Fungicide resistance in *L. maculans* has not been reported in any other canola-growing region, which raises the question of why such differences occur between Australian populations and those in the rest of the world. One possible explanation is

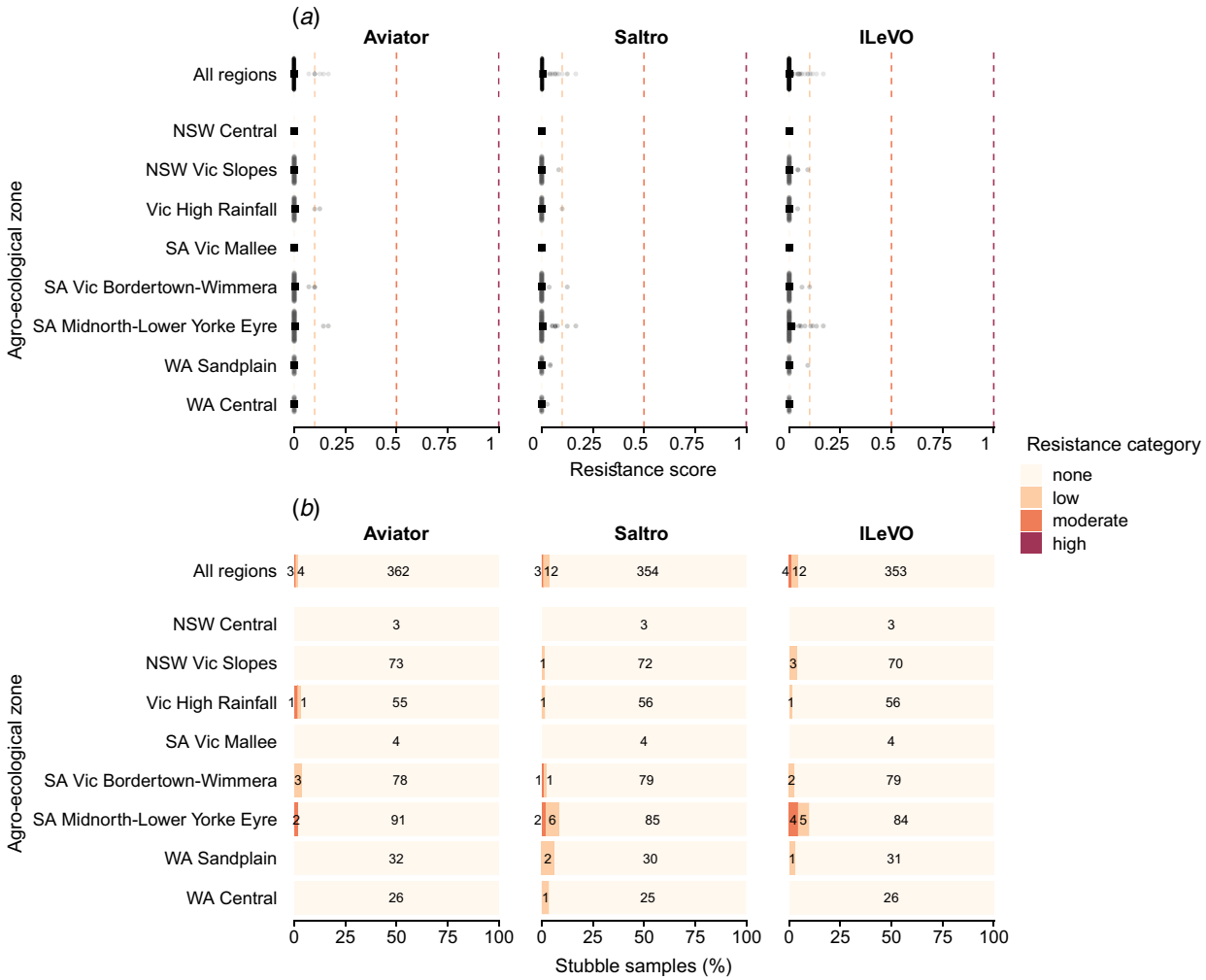


**Fig. 5.** Resistance scores (RS) for 369 stubble-borne *L. maculans* populations to the three SDHI-containing fungicide treatments, for each of eight GRDC agro-ecological zones as well as across all sample locations. (*a*) Individual RS values for each sample (grey points), with a zone-wide mean (black square) and 95% confidence interval of the mean (black line). Upper RS limits for each of the four resistance categories are marked with a coloured dashed line. (*b*) The proportion of samples per zone falling into one of four resistance categories: none (RS = 0), low (RS >0 and ≤0.1), moderate (RS >0.1 and ≤0.5) and high (RS >0.5). The number of samples within each category (if >0) is indicated by the number in each bar segment.

the heavy reliance on fungicides to help minimise the impact of blackleg in Australia, with >95% of growers using at least one fungicide every year, and 50% considering foliar applications depending on the seasonal conditions (Van de Wouw *et al.* 2021). This contrasts with other countries such as Canada, where fungicides for blackleg control have been shown to give limited yield returns and therefore are not used extensively (Zhang and Fernando 2018; Peng *et al.* 2020). An alternative explanation is the potential lack of screening for fungicide resistance in other countries as well as the differences in methods being used. There are only three reports regarding screening for fungicide resistance outside Australia, and those studies were looking at strobilurin sensitivity rather than DMI resistance (Eckert *et al.* 2010; Liu *et al.* 2013; Fraser *et al.* 2017). Furthermore, those reports used standard *in vitro* assays whereby only a small number (~100) of isolates were screened, whereas the *in planta* assays used in the Australian surveys screen thousands of ascospores (Van de Wouw *et al.* 2017).

The present study revealed a lower frequency of resistance to Prosaro® in field populations than the other DMI fungicides. A possible explanation is that DMI-resistance mutations in the field may confer less resistance to prothioconazole than to other DMI actives, and therefore, Prosaro® provides greater protection than the other DMIs. Consistent with this, some isolates with mutations in the promoter of the *ERG11/CYP51* gene described by Yang *et al.* (2020) are much more resistant to tebuconazole, fluquinconazole and flutriafol than to prothioconazole in *in vitro* growth assays.

Foliar fungicide decisions are made during the growing season, and are therefore dependent on environmental conditions. On the other hand, fungicide applications at

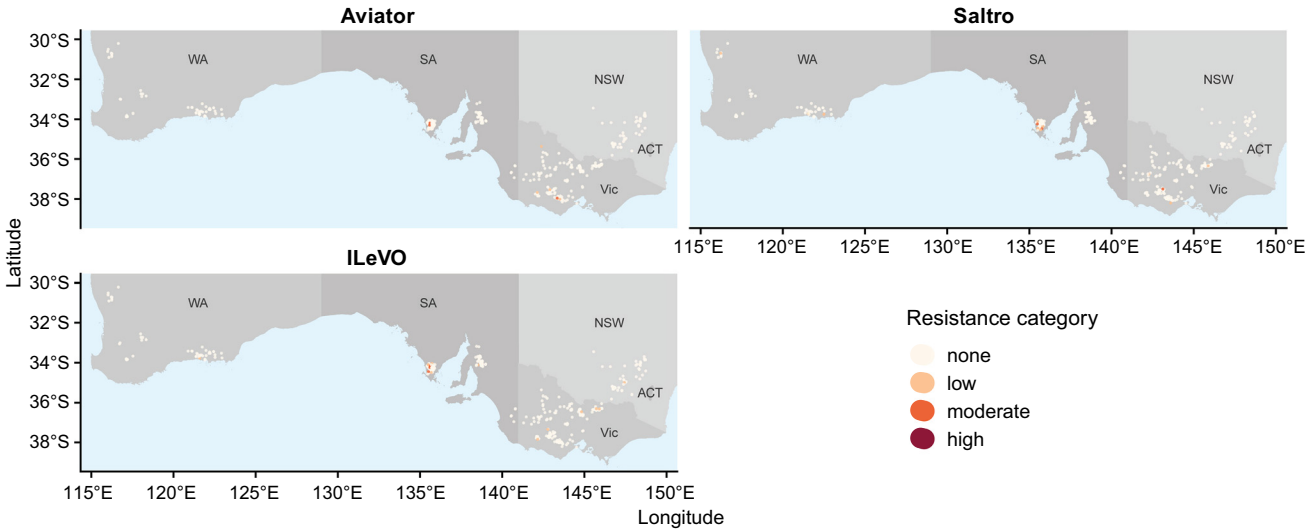


**Fig. 6.** Map of canola stubble sample locations of origin within Australia, with sample icons coloured by their resistance category for each of the three SDHI-containing fungicide treatments. If exact sample coordinates are not known, the position of the point has been randomly moved 0–0.04 units away from the centre of the closest town, to avoid overplotting. WA, Western Australia; SA, South Australia; NSW, New South Wales; Vic, Victoria; ACT, Australian Capital Territory. Resistance categories: none (RS = 0), low (RS >0 and ≤0.1), moderate (RS >0.1 and ≤0.5), and high (RS >0.5).

sowing are made before growers have any knowledge of seasonal conditions and are therefore applied as an insurance. Although the foliar fungicides have been more recently released, our data show that, in 2019, >50% of growers applied at least one foliar fungicide application. This high use of foliar fungicides suggests that there should be selection for resistance in the population. However, it is not yet known whether the use of foliar fungicides results in the same bottleneck pressure on the population as fungicides applied pre-sowing. The use of pre-sowing fungicides (seed dressing and fertiliser-amended) protects the seedling at the early growth stages (up to the fourth leaf); therefore, any fungicide-resistant isolates will be able to colonise the leaves, grow down the petiole and colonise the hypocotyl, leading to crown canker, and thence be present to undergo sexual reproduction in the crop stubble in the following generation. By contrast, we do not know whether fungicide-resistant isolates selected at the 4–6-leaf stage and 30% bloom stage by a foliar fungicide application at that stage will grow into the crown and contribute to the next generation. As such, does foliar fungicide use select for fungicide resistance as strongly as seedling fungicide use? This lack of knowledge around the epidemiology of the pathogen in terms of when new infections stop contributing to the next generation is a limiting factor when developing fungicide use recommendations, because the timing of fungicide use and therefore the potential for fungicide-resistant isolates to contribute to the next generation will directly impact on recommended management strategies.

We found that resistances to the DMI-only treatments – flutriafol, Prosaro® and Jockey® – were all significantly positively correlated, which is to be expected given their shared mode of action at *ERG11/CYP51*. This is also consistent with the identification of insertions within the promoter region, which affect expression of the *Cyp51*, conferring DMI resistance in *L. maculans* and other fungi such as *Blumeriella jaapii* and *Aspergillus fumigatus* (Ma *et al.* 2006; Yang *et al.* 2020; Garcia-Rubio *et al.* 2021). From the significant correlation between the DMI fungicides, one could predict that the detectable frequency of resistance to Prosaro® should be higher in Australian populations than was detected in the present study. This lower-than-expected frequency of resistance to Prosaro® may be due to this fungicide being a mixture of tebuconazole and prothioconazole. Yang *et al.* (2020) found that the 50% effective concentration ($EC_{50}$) and resistance factors (RFs) were much lower for prothioconazole (average RF 2.36) than tebuconazole (average RF 3.99) in DMI-resistant isolates, suggesting that the prothioconazole may be the main contributor to activity in Prosaro®. An alternative explanation is that molecule-specific resistance mechanisms that do not involve *ERG11/CYP51*, such as detoxification or efflux mechanisms (reviewed by Price *et al.* 2015), may also be conferring resistance to particular actives in some Australian populations. Consistent with this scenario is the existence of isolates that do not harbour any mutations in *ERG11/CYP51* and yet display both *in planta* and *in vitro* resistance (Yang *et al.* 2020). Additionally, we found that a small number of populations had high levels of resistance to one DMI-only treatment but no detectable resistance to another, which could also be due to molecule-specific resistance mechanisms that remain to be confirmed through *in vitro* screening of individual isolates.

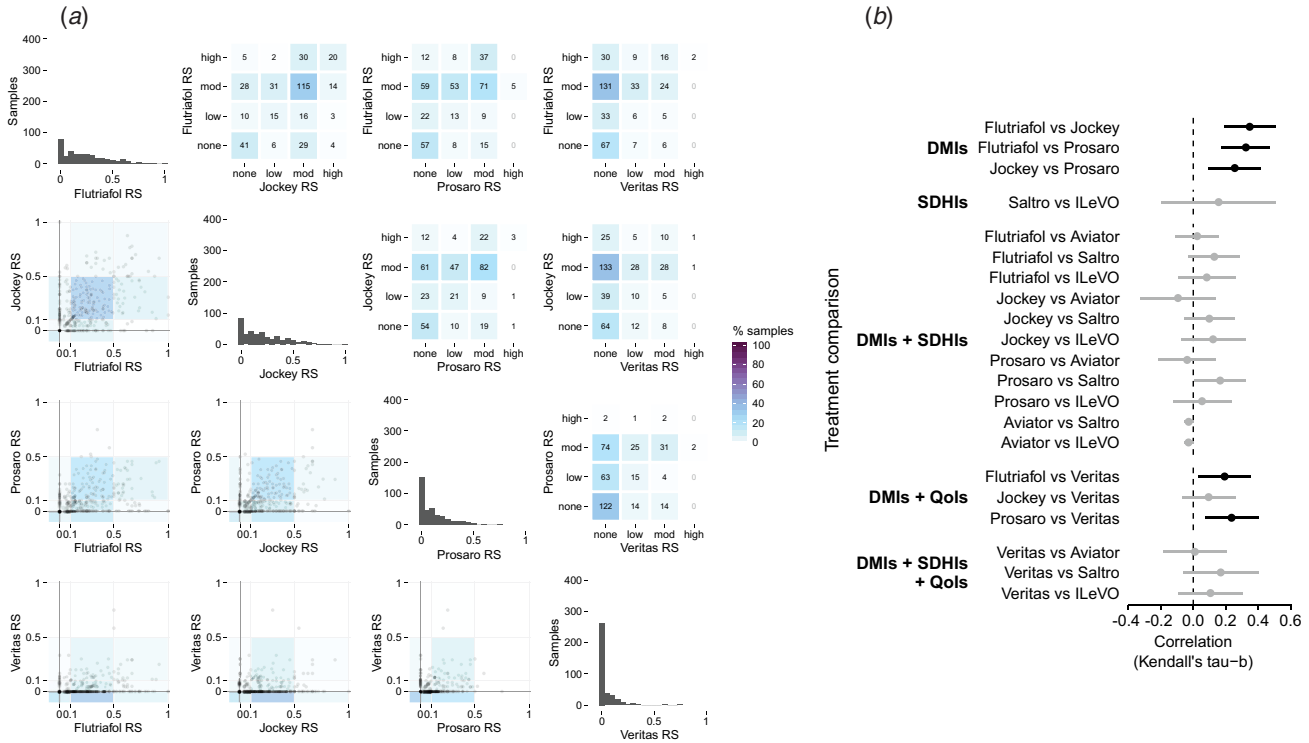


**Fig. 7.** Pairwise comparisons of fungicide treatment resistance scores (RS)/categories from the *in planta* screen. (*a*) A matrix of resistance comparisons for the DMI-containing fungicide treatments. Scatterplots are presented (bottom left) of RS values for each treatment combination, where each grey point is a stubble sample population; background rectangles corresponding to the resistance category combinations are coloured by the proportion of samples that fall into each category combination; combinations that include the no resistance category (RS = 0) are placed to the left/below the relevant axis to enable visualisation. Histograms (on the diagonal) show the distribution of RS values across the samples for each treatment. Heatmaps (top right) show the number of samples falling into each resistance category combination. (For complete matrix of treatment combinations, including the three SDHI-containing treatments, see Fig. S2.) (*b*) Kendall's tau-b correlation coefficients for all pairwise comparisons of resistance categories between treatments, displayed as point estimates (filled circles) and 95% confidence intervals (adjusted for 21 comparisons). Estimates that are not statistically significant (Table S2) are shaded grey.

Although resistance to the DMI fungicides was identified across Australian *L. maculans* populations, the proportion of fungicide-resistant isolates within each population remains unknown. A fruitful avenue of future research in this area may be to develop a molecular method for determining the presence and frequencies of DMI-resistance alleles in the populations. Furthermore, it is unknown how the resistance found in this study directly relates to efficacy of these fungicides in the field. Yang *et al.* (2020) found that the mean RFs of the DMI-resistant isolates ranged from only 2.36 to 5.12. These RFs are similar to those of other species (RF 6–11) in which mutations in the *ERG11/CYP51* promoter region confer resistance (Cools *et al.* 2012; Mair *et al.* 2020), but are relatively low compared with species where resistance is conferred by amino acid changes in the ERG11/CYP51 protein (RF 30–65; Poloni *et al.* 2021). Yield losses associated with high RF values have been determined for *Zymoseptoria tritici* in Europe (Jørgensen *et al.* 2021), and yet little research has been done relating these lower RFs, as seen in *L. maculans*, to fungicide efficacies in the field.

This study explored resistance to Veritas®, a DMI + $Q_OI$ (tebuconazole + azoxystrobin) fungicide, which, at the time of study, was registered on canola only for the control of Sclerotinia stem rot, not blackleg. Despite Veritas® not being directly used to control blackleg, we found evidence of resistance to the treatment across Australian *L. maculans* populations, with two populations (0.5%) having high resistance scores and 51 (14%) having moderate resistance scores. This possibly reflects inadvertent selection for Veritas® resistance in *L. maculans* from exposure to the treatment in the field. Of concern, reduced sensitivity to Veritas® in the Vic High Rainfall agro-ecological zone, which exhibited the highest average levels of resistance in the study, may be increasing in prevalence (Figs S2 and S3). However, no isolates collected from Veritas®-treated seedlings were confirmed as having resistance to Veritas®, although they were resistant to at least one DMI, suggesting that the high levels of resistance may be due to DMI resistance and not $Q_OI$ resistance. Future monitoring of

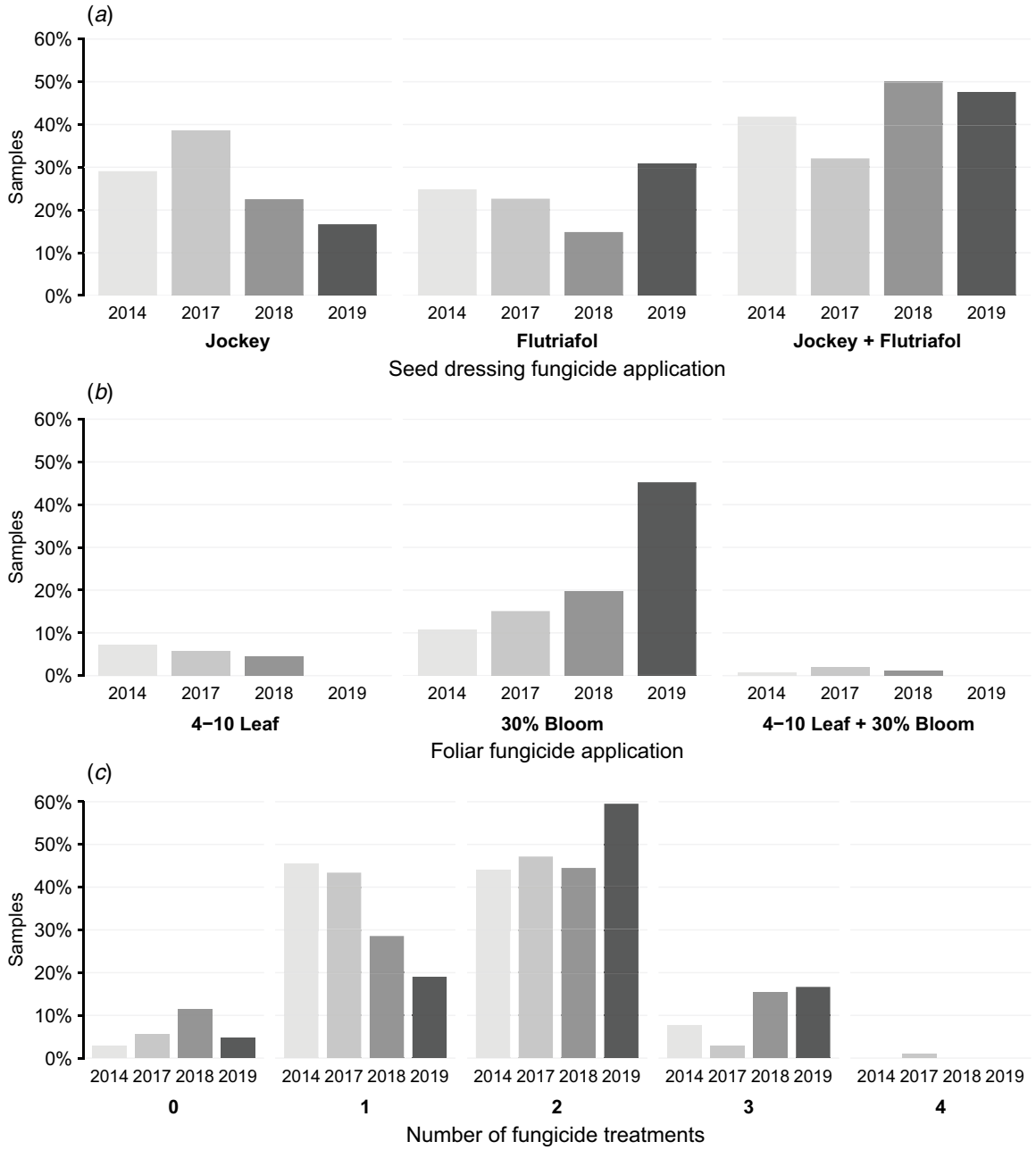


**Fig. 8.** Fungicide use data associated with 330 agronomist-submitted stubble samples from the use-years of 2017 ($n$ = 106), 2018 ($n$ = 182) and 2019 ($n$ = 42). Samples that were not submitted by agronomists ($n$ = 39) or had no associated fungicide use data ($n$ = 29) were not included in the analysis. Stubble samples ($n$ = 141) from use-year 2014 (previously described by Van de Wouw *et al.* 2017) were also included in the analysis. (*a*) Fungicide treatments used at the seed-dressing stage (on the seed/in-furrow). (*b*) Foliar fungicide treatments used at the 4–10-leaf stage, the 30% bloom stage, or both. (*c*) Total number of fungicide treatments used on the crop.

Veritas® resistance in this region may be prudent given the recent registration of Veritas® Opti for blackleg control.

We also found that resistance to Veritas® was significantly positively correlated with resistance to either flutriafol or Prosaro®, suggesting that there may be positive cross-resistance between these three fungicide treatments. The lack of a significant correlation between Veritas® and Jockey® could be due to DMI-specific resistance mechanisms, as previously discussed, or to the inherent stochasticity of the screening method employed here. A reasonable hypothesis is that pre-existing, widespread resistance to DMI fungicides in Australia, as evidenced here

and elsewhere (Van de Wouw *et al.* 2017; Yang *et al.* 2020), has allowed for the evolution of resistance to Veritas® by selecting for $Q_O$I-resistance alleles arising in a DMI-resistant genetic background. Future work should attempt to identify *L. maculans* isolates that are resistant to both DMI and $Q_O$I fungicides, and compare possible variant cytochrome b alleles with those known to confer $Q_O$I resistance in other fungi (Fernández-Ortuño *et al.* 2008).

A limitation of the methodology is the lack of biological replication of the inoculum source, in addition to sampling of the stubble whereby the same paddocks cannot be sampled in consecutive years because canola is grown in rotation. However, it is noteworthy that isolates with fungicide resistance were identified from a subset of populations, suggesting that the resistance that is being detected is biologically relevant. Unlike the DMI-only fungicides, we found a widespread lack of resistance to SDHI fungicides in Australian *L. maculans* populations, with moderate resistance scores detected in only 14 populations (3.8%). Whether the non-zero resistance scores found in some populations are the result of real but rare SDHI-resistant genotypes or due to technical limitations of the *in planta* assay (see below) is unclear. Given that SDHI resistance in fungal phytopathogens overwhelmingly results from point mutations in the *sdhB*, *sdhC* and *sdhD* target genes (Sierotzki and Scalliet 2013), it is possible that a small fraction of standing variation in these genes confers SDHI resistance in *L. maculans* prior to selection. Alternatively, given that little is known about the spectrum of SDHI action against *L. maculans*, the small number of populations with higher RS scores observed in this study may be due to a small number of isolates with reduced sensitivity, but not fully resistant isolates, that may still be able to grow, albeit slowly, in the presence of the fungicide, and therefore still colonise the plant. Notably, three stubble-borne populations – all within the SA Midnorth-Lower Yorke Eyre agro-ecological zone – had non-zero RS values for both Saltro® and ILeVO®, suggesting that they have the highest chance of harbouring real SDHI-resistance or -tolerance alleles. This region of Australia should be monitored closely for the emergence of SDHI resistance; pro-active surveillance of variation at the *sdhB/C/D* genes in South Australia, and the rest of the country, may be able to detect selection of SDHI-resistance alleles before efficacy of the fungicide treatments in the field is reduced. Interestingly, this region of Australia was also the first for detection of SDHI fungicide resistance for net blotch in barley (Ireland *et al.* 2021).

We hypothesised that high-rainfall regions, which equate to higher canola intensity and therefore higher fungicide use, would be more likely to have populations with greater resistance scores, indicating fungicide resistance; however, the dataset shows significant differences only for the Vic High Rainfall agro-ecological zone. The lack of more significant findings probably reflects that whole-farming practices, and not just the individual paddock practices, are contributing to the selection of fungicide resistance in *L. maculans*. This is consistent with recent modelling studies in wheat, focusing on *Z. tritici*, that have shown the major driver influencing the evolution of fungicide resistance to be fungicide use at the regional scale, not the local scale (Garnault *et al.* 2021). In Australia, fungicides such as Prosaro® are registered for blackleg and Sclerotinia in canola, as well as pathogens in wheat and barley. As such, the canola stubble that remains in the paddock at the end of the growing season may be exposed to the same fungicides in the following year if a wheat or barley crop is sown. The impact of these farming practices on the selection of pathogens is unknown.

The *in planta* assay used in this study has notable advantages and limitations. Given that the assay screens for disease on plants by using populations of *L. maculans* (potentially hundreds of thousands of ascospores per population) and also applies fungicide treatments in a manner similar to that in the field, the results likely reflect expected fungicide-resistant disease pressure in the field. This is not always possible with *in vitro* screens that rely on a small number of isolates or are unable to measure the impact of the plant and environment on efficacy (e.g. Eckert *et al.* 2010; Sewell *et al.* 2017). Similarly, the large number of individual ascospores being screened increases the chances of detecting highly resistant but rare genotypes. Additionally, DMI-resistant isolates have been recovered from DMI-resistant populations identified from this screen (Yang *et al.* 2020; data not shown), indicating that the *in planta* method is detecting resistant genotypes. However, we note that Veritas®-resistant or SDHI-resistant isolates have yet to be obtained from any of the populations screened in the study.

A possible limitation of the *in planta* assay as implemented here is that fungicide application, as it is in the field, may be slightly uneven across individual seeds or leaves, leading to localised reductions in fungicide concentration and permitting some non-resistant ascospores to form leaf lesions. Such 'application stochasticity' could explain the very low frequency of disease seen on SDHI-treated plants in this study, even though SDHI-resistant isolates were not detected through lesion culturing; however, the existence of rare SDHI-resistant genotypes cannot be ruled out. Additionally, the data generated by this study, using normalised resistance scores between 0 and 1, contain a high proportion of 0 values for many fungicide treatments. This makes correlations between treatments difficult to analyse statistically, because the presence of 0 values for both treatments in a comparison is not informative towards cross-resistance trends in whole populations. Our data seemingly supported small but significant negative correlations between Aviator® XPro resistance and both ILeVO® and Saltro® resistance, as well as a positive correlation between Prosaro® resistance and Saltro® resistance, all of which are likely spurious given prior knowledge. The small number of non-zero values for one or both treatments in these comparisons appears to have

reduced the effective sample size, leading to inaccurate correlational conclusions despite significant *P*-values. In general, the resistance correlations presented in this study should be treated as tentative. We were also unable to explore substantially the differences in fungicide resistance between geographical regions and years, because our stubble samples were largely voluntarily submitted by growers, and the sampling therefore reflects only a subsection of all *L. maculans* populations across Australia and only the period 2018–20. As such, any geographical or temporal trends in the data, including the statistically significant changes in Veritas resistance, should also be treated as tentative.

Overall, this study indicates that in Australian populations of *L. maculans*, DMI resistance is widespread, whereas $Q_OI$ + DMI resistance is less common and SDHI resistance is rare. The presence of detectable resistance to Veritas ($Q_OI$ + DMI) at high levels in two individual populations suggests that the use of the newly registered Veritas® Opti fungicide treatment may be less effective for blackleg control than expected in some regions. Similarly, the newly registered treatment Maxentis® EC (azoxystrobin + prothioconazole) may also have reduced efficacy in regions with high pre-existing Veritas® resistance. By contrast, the widespread lack of SDHI resistance despite the use of Aviator® XPro since 2016 suggests no such limitation for the newly introduced Saltro® and ILeVO® fungicide treatments, although low levels of resistance in some populations raise the possibility of rare SDHI resistance alleles being selected for as usage of these treatments grows over time.

This widescale survey provides a benchmark for monitoring of fungicide resistance to these new chemistries and will possibly allow the tracking of potential emergence of resistance well before it would be detected through field-failure of the fungicide. This provides a potentially powerful model system through which to witness the rise of fungicide resistance, rather than tracking it retroactively or on a relatively small number of isolates tested under *in vitro* conditions, which happens often in other plant disease systems. Future experiments should continue to monitor fungal populations for changes in the efficacy of fungicides, using methods developed previously and in this research, as well as genotyping of populations for mutations associated with fungicide resistance.

## Supplementary material

Supplementary material is available online.

## References


Bartlett DW, Clough JM, Godwin JR, Hall AA, Hamer M, Parr-Dobrzanski B (2002) The strobilurin fungicides. *Pest Management Science* **58**, 649–662. doi:10.1002/ps.520

Cools HJ, Bayon C, Atkins S, Lucas JA, Fraaije BA (2012) Overexpression of the sterol 14α-demethylase gene (*MgCYP51*) in *Mycosphaerella graminicola* isolates confers a novel azole fungicide sensitivity phenotype. *Pest Management Science* **68**, 1034–1040. doi:10.1002/ps.3263

Eckert MR, Rossall S, Selley A, Fitt BDL (2010) Effects of fungicides on *in vitro* spore germination and mycelial growth of the phytopathogens *Leptosphaeria maculans* and *L. biglobosa* (phoma stem canker of oilseed rape). *Pest Management Science* **66**, 396–405. doi:10.1002/ps.1890

Fernández-Ortuño D, Torés JA, de Vicente A, Pérez-García A (2008) Mechanisms of resistance to QoI fungicides in phytopathogenic fungi. *International Microbiology* **11**, 1–9.

Fisher MC, Hawkins NJ, Sanglard D, Gurr SJ (2018) Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science* **360**, 739–742. doi:10.1126/science.aap7999

Fitt BDL, Brun H, Barbetti MJ, Rimmer SR (2006) World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). *European Journal of Plant Pathology* **114**, 3–15. doi:10.1007/s10658-005-2233-5

Fraser M, Hwang S-F, Ahmed HU, Akhavan A, Stammler G, Barton W, Strelkov SE (2017) Sensitivity of *Leptosphaeria maculans* to pyraclostrobin in Alberta, Canada. *Canadian Journal of Plant Science* **97**, 83–91. doi:10.1139/cjps-2015-0382

Garcia-Rubio R, Gonzalez-Jimenez I, Lucio J, Mellado E (2021) *Aspergillus fumigatus* cross-resistance between clinical and demethylase inhibitor azole drugs. *Applied and Environmental Biology* **87**, e02539-20. doi:10.1128/AEM.02539-20

Garnault M, Duplaix C, Leroux P, Couleaud G, David O, Walker A-S, Carpentier F (2021) Large-scale study validates that regional fungicide applications are major determinants of resistance evolution in the wheat pathogen *Zymoseptoria tritici* in France. *New Phytologist* **229**, 3508–3521. doi:10.1111/nph.17107

Hammond KE, Lewis BG, Musa TM (1985) A systemic pathway in the infection of oilseed rape plants by *Leptosphaeria maculans*. *Plant Pathology* **34**, 557–565. doi:10.1111/j.1365-3059.1985.tb01407.x

Hollomon DW (2015) Fungicide resistance: facing the challenge: a review. *Plant Protection Science* **51**, 170–176. doi:10.17221/42/2015-PPS

Ireland KB, Beard C, Cameron J, Chang S, Davidson JA, Dodhia KN, Garrard TA, Hills AL, Hollaway G, Jayasena KW, Kiss L, Mair WJ, Marcroft SJ, McLean MS, Milgate A, Poole N, Simpfendorfer S, Snyman L, Thomas GJ, Wallwork H, Van de Wouw AP, Zulak KG, Lopez-Ruiz FJ (2021) 'Fungicide resistance management in Australian grain crops', (Grains Research and Development Corporation: Canberra, ACT, Australia) https://grdc.com.au/fungicide-resistance-management-in-australian-grain-crops. [Accessed August 2021]

Jørgensen LN, Matzen N, Heick TM, Havis N, Holdgate S, Clark B, Glazek M, Blake J, Korbas M, Danielewicz J, Maumene C, Rodemann B, Weigand S, Kildea S, Bataille C, Brauna-Morževska E, Gulbis K, Ban R, Berg G, Semaskiene R, Stammler G (2021) Decreasing azole sensitivity of *Z. tritici* in Europe contributes to reduced and varying field efficacy. *Journal of Plant Diseases and Protection* **128**, 287–301. doi:10.1007/s41348-020-00372-4

Joseph-Horne T, Hollomon DW (1997) Molecular mechanisms of azole resistance in fungi. *FEMS Microbiology Letters* **149**, 141–149. doi:10.1111/j.1574-6968.1997.tb10321.x

Liu C, Fernando DWG, Gan YT, Kutcher HR, Peng G (2013) Baseline sensitivity of *Leptosphaeria maculans* to strobilurin fungicides. *Canadian Journal of Plant Pathology* **35**, 119.

Ma Z, Proffer TJ, Jacobs JL, Sundin GW (2006) Overexpression of the 14α-demethylase target gene (*Cyp51*) mediates fungicide resistance in *Blumeriella jaapii*. *Applied and Environmental Microbiology* **72**, 2581–2585. doi:10.1128/AEM.72.4.2581-2585.2006

Mair WJ, Thomas GJ, Dodhia K, Hills AL, Jayasena KW, Ellwood SR, Oliver RP, Lopez-Ruiz FJ (2020) Parallel evolution of multiple mechanisms for demethylase inhibitor fungicide resistance in the barley pathogen *Pyrenophora teres* f. sp. *maculata*. *Fungal Genetics and Biology* **145**, 103475. doi:10.1016/j.fgb.2020.103475

Peng G, Liu X, McLaren DL, McGregor L, Yu F (2020) Seed treatment with the fungicide fluopyram limits cotyledon infection by *Leptosphaeria maculans* and reduces blackleg of canola. *Canadian Journal of Plant Pathology* **42**, 480–492. doi:10.1080/07060661.2020.1725132

Poloni NM, Carvalho G, Vicentini SNC, Dorigan AF, Maciel JLN, McDonald BA, Moreira SI, Hawkins N, Fraaije BA, Kelly DE, Kelly SL, Ceresini PC (2021) Widespread distribution of resistance to triazole fungicides in Brazilian populations of the wheat blast pathogen. *Plant Pathology* **70**, 436–448. doi:10.1111/ppa.13288

Price CL, Parker JE, Warrilow AGS, Kelly DE, Kelly SL (2015) Azole fungicides: understanding resistance mechanisms in agricultural fungal pathogens. *Pest Management Science* **71**, 1054–1058. doi:10.1002/ps.4029

R Core Team (2019) R: a language and environment for statistical computing. The R Foundation, Vienna, Austria.

Sewell TR, Hawkins NJ, Stotz HU, Huang YJ, Kelly SL, Kelly DE, Fraaije B, Fitt BDL (2017) Azole sensitivity in *Leptosphaeria* pathogens of oilseed rape: the role of lanosterol 14α-demethylase. *Scientific Reports* **7**, 15849. doi:10.1038/s41598-017-15545-9

Sierotzki H, Scalliet G (2013) A review of current knowledge of resistance aspects for the next-generation succinate dehydrogenase inhibitor fungicides. *Phytopathology* **103**, 880–887. doi:10.1094/PHYTO-01-13-0009-RVW

Signorell A (2021) 'DescToola: tools for descriptive statistics. R package version 0.99.41'. (The R Foundation: Vienna, Austria). https://andrisignorell.github.io/DescTools/; https://github.com/AndriSignorell/DescTools/

Sprague SJ, Balesdent M-H, Brun H, Hayden HL, Marcroft SJ, Pinochet X, Rouxel T, Howlett BJ (2006) Major gene resistance in *Brassica napus* (oilseed rape) is overcome by changes in virulence of populations of *Leptosphaeria maculans* in France and Australia. *European Journal of Plant Pathology* **114**, 33–40. doi:10.1007/s10658-005-3683-5

Sprague SJ, Marcroft SJ, Lindbeck KD, Ware AH, Khangura RK, Van de Wouw AP (2018) Detection, prevalence and severity of upper canopy infection on mature *Brassica napus* plants caused by *Leptosphaeria maculans* in Australia. *Crop & Pasture Science* **69**, 65–78. doi:10.1071/CP17140

Van de Wouw AP, Marcroft SJ, Howlett BJ (2016) Blackleg disease of canola in Australia. *Crop & Pasture Science* **67**, 273–283. doi:10.1071/CP15221

Van de Wouw AP, Elliott VL, Chang S, López-Ruiz FJ, Marcroft SJ, Idnurm A (2017) Identification of isolates of the plant pathogen *Leptosphaeria maculans* with resistance to the triazole fungicide fluquinconazole using a novel *in planta* assay. *PLoS One* **12**, e0188106. doi:10.1371/journal.pone.0188106

Van de Wouw AP, Howlett BJ, Idnurm A (2018) Changes in allele frequencies of avirulence genes in the blackleg fungus, *Leptosphaeria maculans*, over two decades in Australia. *Crop & Pasture Science* **69**, 20–29. doi:10.1071/CP16411

Van de Wouw AP, Marcroft SJ, Sprague SJ, Scanlan JL, Vesk PA, Idnurm A (2021) Epidemiology and management of blackleg of canola in response to changing farming practices in Australia. *Australasian Plant Pathology* **50**, 137–149. doi:10.1007/s13313-020-00767-9

Van de Wouw AP, Marcroft SJ, Howlett BJ (2016) Blackleg disease of canola in Australia. *Crop & Pasture Science* **67**, 273–283.

Wickham H, François R, Henry L, Müller K (2019) dplyr: A grammar of data manipulation. https://CRAN.R-project.org/package=dplyr.

Yang Y, Marcroft SJ, Forsyth LM, Zhao J, Li Z, Van de Wouw AP, Idnurm A (2020) Sterol demethylation inhibitor fungicide resistance in *Leptosphaeria maculans* is caused by modifications in the regulatory region of *ERG11*. *Plant Disease* **104**, 1280–1290. doi:10.1094/PDIS-10-19-2088-RE

Zhang X, Fernando DW (2018) Insights into fighting against blackleg disease of *Brassica napus* in Canada. *Crop & Pasture Science* **69**, 40–47. doi:10.1071/CP16401

Zheng X, Koopmann B, Ulber B, von Tiedemann A (2020) A global survey on diseases and pests in oilseed rape–current challenges and innovative strategies of control. *Frontiers in Agronomy* **2**, 590908. doi:10.3389/fagro.2020.590908

**Data availability.** The data that support this study are available in the article and accompanying online supplementary material.

**Conflicts of interest.** Dr Angela Van de Wouw is an Associate Editor of *Crop and Pasture Science* but was blinded from the peer-review process for this paper.Dr Leanne Forsyth is employed by Syngenta Crop Protection but recused herself from the generation and analysis of fungicide survey data.

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