



Epidemiology and management of blackleg of canola in response to changing farming practices in Australia

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Abstract

The Australian canola industry was established in the 1970s and has expanded since that time, particularly in the last two decades. This review addresses the changes in farming practices since the year 2000 and the epidemiological and management consequences for blackleg, caused by the fungus *Leptosphaeria maculans*, the main disease impacting *Brassica napus* production. To help understand the change in production practices, a survey of over 100 growers and agronomists was conducted. Modern management practices include increased crop residue retention, frequency of canola in crop sequences, number of resistance genes in cultivars, use of hybrids and fungicides as well as earlier sowing and flowering times. While some of the changes identified in the survey and in this review increase the risk and severity of disease, including new symptoms like upper canopy infection, others provide novel strategies for control. Keeping these changes in mind, a set of research priorities towards long term sustainable management of blackleg disease are identified.

Keywords Crop rotation · Fungicides · Grower survey · Integrated disease management · *Leptosphaeria maculans* · Resistance genes

Introduction

Integrated disease/pest management is a widely-adopted philosophy in agriculture that is used to maximise plant yields by a reduction in microbial diseases or animal pests through the implementation of multiple mitigation strategies. The concept was developed in the 1960s by entomologists and later extended to microbial diseases (Jacobsen 1997; Cook 2000). However, these management strategies are not static, in that the release of new technologies in farming systems, plant cultivars or chemicals can require revisions on management. However, the impact of such changes on disease management are rarely assessed.

This paper focuses on the management of *Leptosphaeria maculans* management in Australia, although blackleg disease is a major threat to canola (*Brassica napus*, oilseed rape) production worldwide, except in China (Fitt et al. 2006; Zhang et al. 2014). Blackleg disease results in 10–20% yield losses annually in Canada and the United Kingdom, with up to 90% yield loss caused by epidemics in Australia (West et al. 2001; Fitt et al. 2006; Sprague et al. 2006a; Zhang and Fernando 2018). Blackleg disease is stubble-borne, with sexual reproduction on infected crop residue remaining at the end of the growing season after harvest. The sexual spores are released following rainfall events and land on the cotyledons or leaves of seedlings, germinate and then invade the plant via wounds or stomata (Fig. 1). The fungus then causes necrotic lesions on the leaves and cotyledons before entering a biotrophic phase to allow growth down the petiole and into the stem (Hammond and Lewis 1987). At maturity, the fungus then switches back to a necrotrophic lifestyle and causes blackening of the stem, cankering of plants and, in extreme situations, killing the plant (Hammond et al. 1985).

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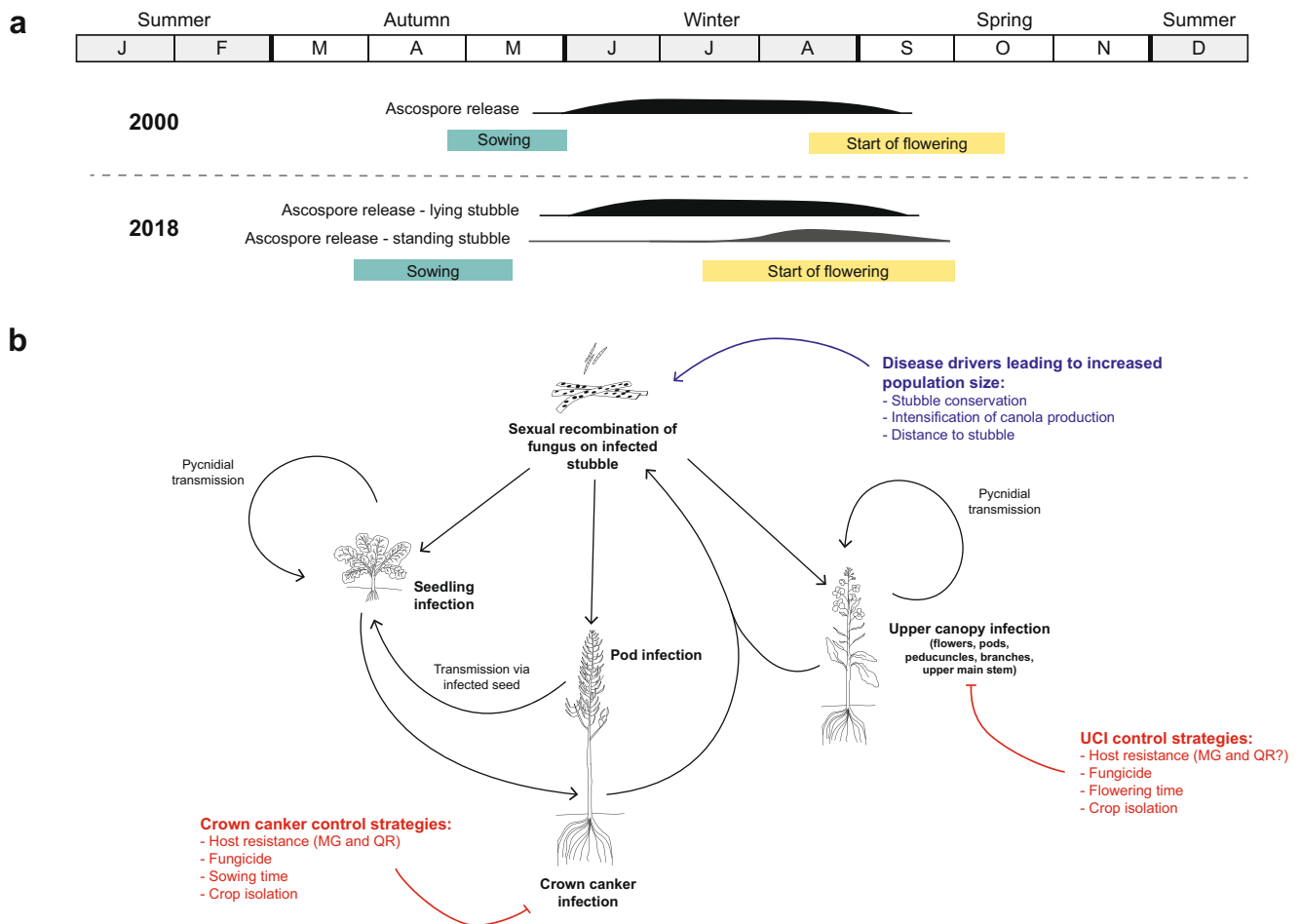


Fig. 1 Impact of changing farming systems on the life cycle of *Leptosphaeria maculans* and control strategies to minimise the impact of blackleg disease. **a** Sowing and flowering times have moved forward significantly in Australia from 2000 compared to 2018, resulting in changes in exposure of spores released from crop residues. **b** The changes in farming practices have led to changes in disease epidemiology of *L. maculans*. Earlier sowing results in seedlings spending less time exposed to spores in the vulnerable growth stage.

In the past 20 years, there have been both scientific and technological advances that have impacted the Australian canola industry to improve production (Fig. 2). Shifts in rainfall patterns have been a major driver for the implementation of many of these changes. The reliability of the autumn ‘break’, the rains on which winter grain crops are sown in southern Australia, has declined particularly during the Millennium drought (1998–2010), but is a trend that is likely to continue (Verdon-Kidd et al. 2014). Adequate moisture at sowing is critical for the successful establishment of small-seeded crops such as canola. There has also been significant reductions in growing season rainfall across the winter cropping regions of Australia since 1970 (www.bom.gov.au/state-of-the-climate/, accessed 13 May 2020). In this review we describe and then investigate via a survey of growers and agronomists, changes in practices associated with canola production in Australia and the related impact on management of *L. maculans*.

Since it is seedling infection that leads to crown canker, earlier sowing times will potentially reduce the severity of crown canker. In contrast, earlier flowering times have resulted in the flowers, pods and branches of plants now being exposed to spores, leading to the development of symptoms termed upper canopy infection (UCI). Highlighted in red are control strategies known to minimise different aspects of disease, as well as the disease drivers leading to increased disease pressure. MG, major gene resistance; QR, quantitative resistance

Survey to assess perceptions about changing farm practices

In addition to assessing published literature and government reports, we conducted a survey to determine the extent of change to identify: (i) how blackleg disease management strategies used by industry have changed over time; and (ii) areas of blackleg research to deliver updated management advice relevant to current farming practices.

A survey consisting of 30 questions was conducted using the online survey platform, Survey Monkey (www.surveymonkey.com.au), designed to determine which factors in canola and blackleg management have changed between 2000 and 2018. Respondents were asked general questions including whether they are a farmer or agronomist/advisor, the postcode of the location of their own or their client’s primary property, size of that primary property and long-

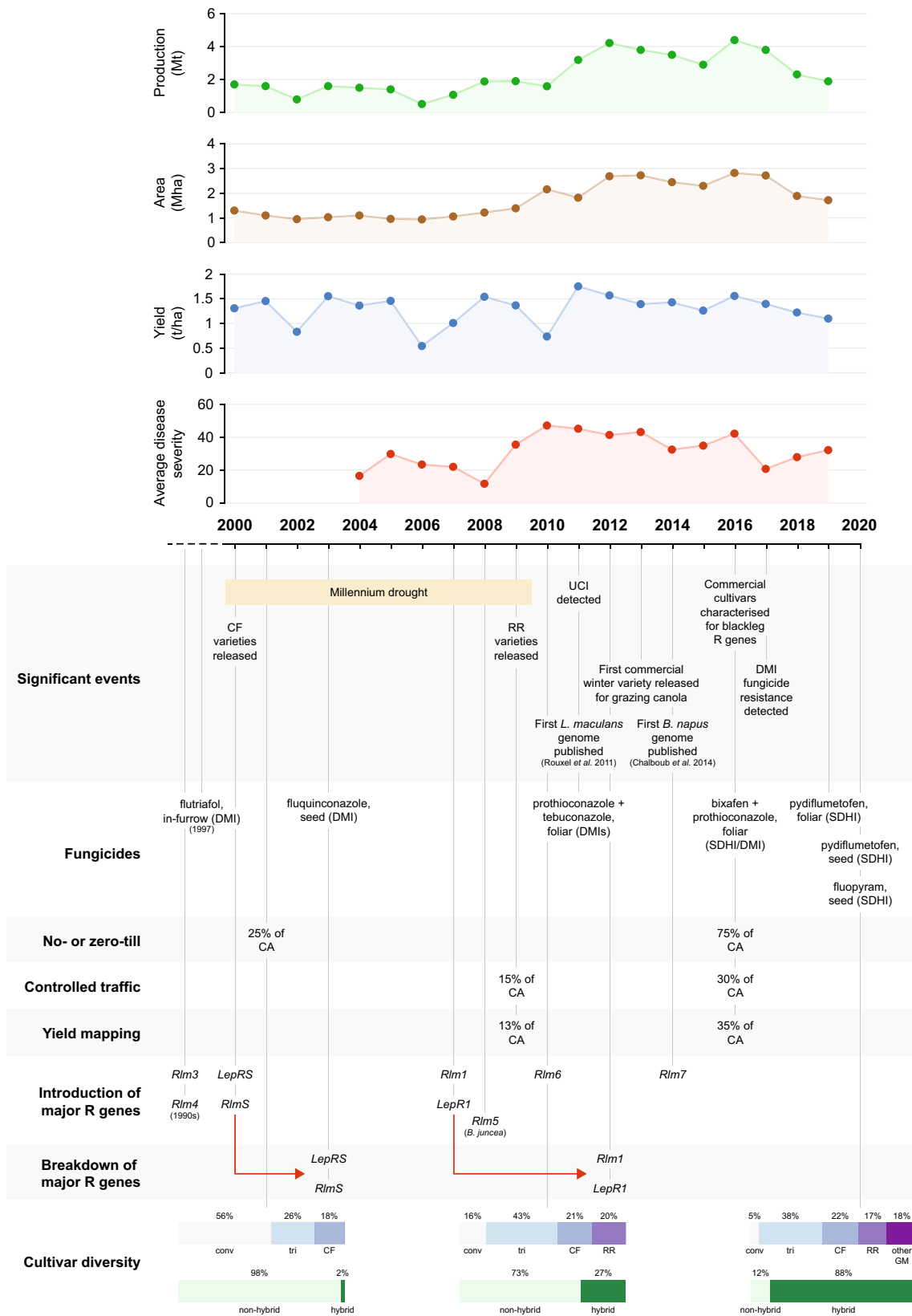


Fig. 2 Summary of significant events in Australian canola production including the introduction new technologies, as well as changes in farming practices and cultivar variety. Decreased production in the early 2000s reflects the Millennium drought. R, resistance; CF,

Clearfield®; RR, Roundup Ready®; UCI, upper canopy infection; CA, cropping area; conv, conventional; tri, triazine tolerant; GM, genetically modified

term average annual growing season rainfall (April through October), which was used to categorise responses into low (<250 mm rainfall), medium (250–400 mm) or high (>400 mm) rainfall regions. For most of the questions, the respondents were asked to answer each in relation to historic on-farm practices (the year 2000 was selected as a guide to the timeframe) and current practices (2018). A total of 112 respondents, representing all canola-growing regions of Australia, participated in the survey. Of the 112, 74 respondents were advisors who provide advice to multiple canola growers within their regions.

The responses from survey respondents are ordered categorical data, e.g. 0–10%, 11–20%, 21–30%, >30% farm sown with canola. That is, the data are not continuous, but are classes arrayed in order and therefore the appropriate analyses are ordinal regression (Agresti 2010). These are related to linear regression and analysis of variance and other generalised linear models in that a linear model is written consisting of explanatory factors (or covariates) and error terms. However, conversion to a categorical (cf. continuous) response is made via the cumulative link model, using a logit link. This is akin to fitting models of the probability of exceeding a given threshold between successive classes, e.g. the probability of 11–20% rather than 0–10% canola. For each question, a series of models were fitted with a set of covariates: State of Australia, Year (2000 or 2018) and growing season rainfall (centered on median). Random effects of respondent ensured the analyses were paired in all cases. The following cumulative log odds mixed effect models were fitted with combinations of three covariates which were compared using Akaike's Information Criteria and Likelihood Ratios; significance was determined through Chi-square tests. The models compared included a null model (just respondent), Year, Year + State, Year + Rainfall, Year + Rainfall + State. Two models were fitted with interactions with Year: Year + Rainfall + Year × Rainfall and; Year + Rainfall + Year × State. Rainfall and State are somewhat confounded, so we did not fit a model for all three terms plus interactions with Year. Analyses were run with the package “ordinal” (Christensen 2019) in the R software environment (Team 2019). For a summary of the survey questions and the statistical analysis see Supplementary File 1.

Intensification of production

Canola is one of the major oilseed crops worldwide. In 2000/2001, global production was 36.7 Mt, harvested from an area of 23.9 Mha (Gunstone 2004), and by 2017/2018 the area had doubled to 74.0 Mt from an area of 31.3 Mha (Van de Wouw and Howlett 2020). Globally yields have increased from 1.5 t/ha to 2.4 t/ha over the past 20 years (Van de Wouw and Howlett 2020; Fischer et al. 2014). In Australia

specifically, canola production has also doubled in the past 20 years (Fig. 2), increasing from 1.8 Mt in 2000/2001 to 3.8 Mt in 2017/2018, with a peak of 4.4 Mt in 2016/2017; however, yields have fluctuated seasonally (Gunstone 2004; Van de Wouw and Howlett 2020). Whilst increased canola production globally has been driven by both increased area and improved yields, in Australia the increase has mainly been driven by an increase in area (Fig. 2). The increase in area sown to canola means that canola is now the third largest crop in Australia, worth AU\$2.1 billion in 2017–18 (www.abs.gov.au), and has spread into lower rainfall regions rather than the typical high rainfall areas (Zhang et al. 2016a). Grower survey data also reflect the intensification of canola with respondents reporting a statistically significant increase in area sown to canola, from <10% of the farm area in 2000 to >30% in 2018 (Fig. 3a).

One potential factor driving canola intensity in Australia in the past 20 years is the increased diversity in commercially available cultivars in terms of herbicide resistance. According to canola cultivar lists published in the official blackleg rating guides produced by the Grains Research and Development Corporation (www.grdc.com.au/GRDC-FS-blacklegManagementGuide), over the past two decades the number of cultivars commercially available has almost doubled from 38 in 2001 to 60 in 2020, with large changes in available herbicide resistance (Fig. 2). The proportion of conventional cultivars (those lacking specific herbicide resistance) dropped from 50% to 5% of the available cultivars between 2001 and 2020. This large change was initially driven by the introduction of the non-genetically manipulated Clearfield® and triazine-tolerant varieties, and then by the introduction of the Round-up Ready® technology which provides new weed management options; by 2010, 27% of cultivars were harbouring this technology (Fig. 2). In 2020, the second-generation Round-up Ready® technology - termed TruFlex® - was released, which extends the glyphosate application windows for farmers providing extended opportunities for controlling weeds. In addition, herbicide stacks are also available within the same cultivars: Clearfield® and triazine-tolerance; Round-up Ready® and triazine-tolerance; TruFlex® and Clearfield®; and TruFlex® and triazinotolerance. The GM-herbicide technologies now represent almost 40% of the commercially available cultivars and are likely to increase due to the extended opportunities for weed control.

Other advances in cultivar diversity include hybrid canola varieties, winter dual-purpose varieties and high stability oil varieties. Hybrid canola varieties are generally considered more vigorous and weed competitive and have been shown to out-yield open-pollinated cultivars by up to 20% in both Australia and Canada, depending on rainfall and growing season length (Zhang et al. 2016a; Brandt et al. 2007). Consequently, hybrid cultivars have increased from 2% of

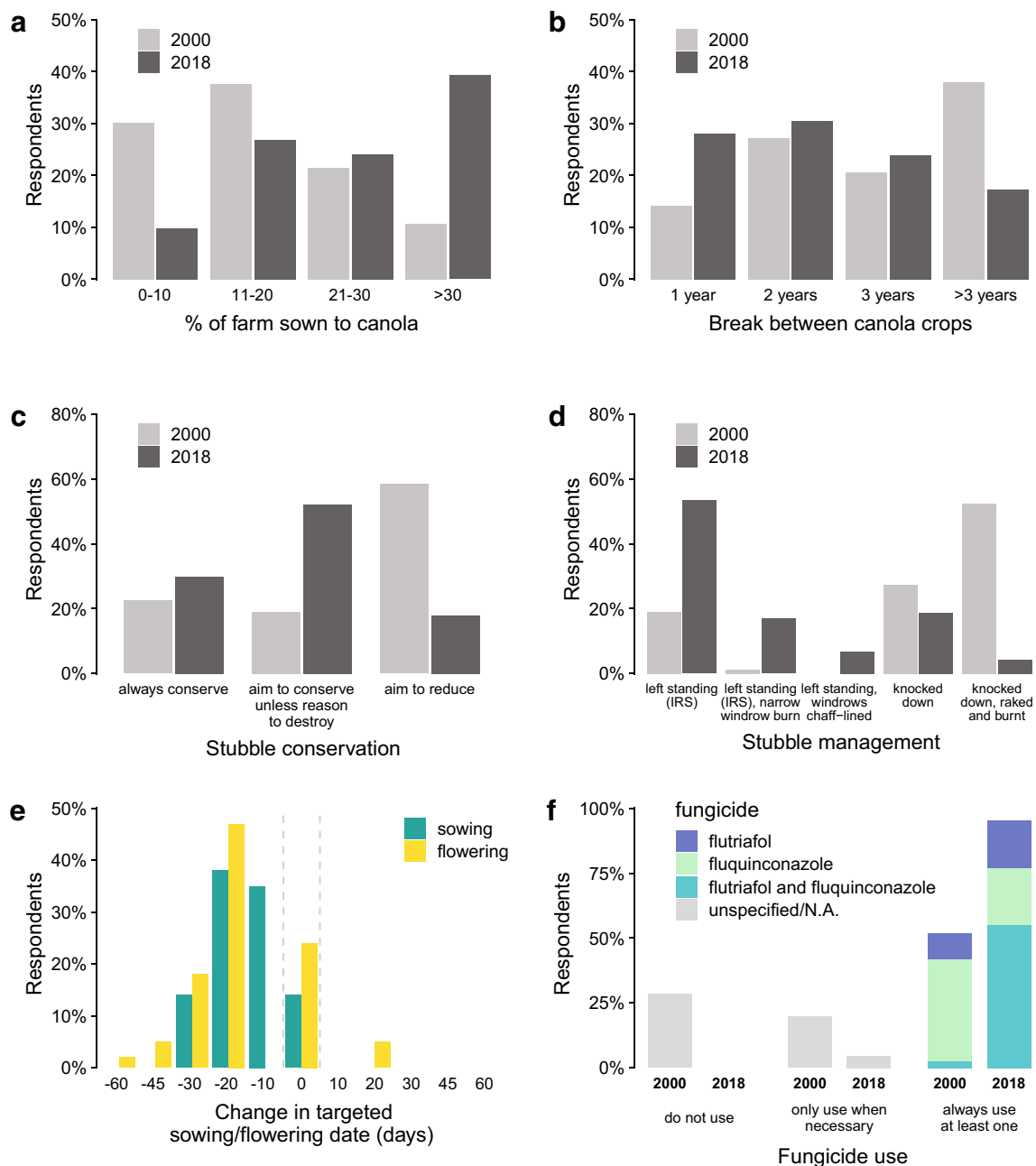


Fig. 3 Results from grower survey in relation to **a** canola intensity, **b** cropping sequences/rotation, **c** stubble conservation, **d** stubble management, **e** changes in sowing and flowering times between 2000 and 2018, and **f** fungicide use. IRS, inter-row sowing

the commercially available cultivars in Australia in 2001 to 88% in 2020 (Fig. 2). Despite the majority of commercially available cultivars being hybrids, according to the respondents in our grower survey, 43% grew open pollinated cultivars in 2018 whilst 57% grew hybrids. The popularity of the open pollinated cultivars probably reflects the high cost of hybrid seed (~\$30/kg) and the ability to retain seed from the open pollinators (Zhang et al. 2016a).

The release of winter varieties underpinned the widespread and rapid adoption of canola for dual-purpose production in higher-rainfall environments in mixed farming enterprises

(crop and livestock) with increases in whole farm productivity of up to \$100 per ha (Kirkegaard et al. 2016a; Sprague et al. 2015; Bell et al. 2015). Dual-purpose refers to the grazing of established crops by livestock during vegetative growth in late autumn and winter, with the subsequent recovery of the crop to produce grain after the removal of animals, often with minimal impact on grain yield compared to an ungrazed crop (Kirkegaard et al. 2012). The availability of winter cultivars allows early sowing opportunities to capture maximum forage production as crops remain vegetative until after winter (Lilley et al. 2015). As all canola cultivars can be grazed and are a

high-quality source of feed, dual-purpose production is being adopted across all rainfall zones.

As canola stubble is the inoculum source for the following season, reduced crop rotation coupled with increased canola production results in higher inoculum loads the following year. Tighter canola rotations in Canada and Germany decreased yield potentials and increased blackleg disease severity (Harker et al. 2015; Hegewald et al. 2017). Recent studies in Australia suggest that blackleg could be a contributing factor to the large gaps between achieved and potential grain yield of canola. An analysis of farm yields between 1998 and 2015 found yields were between 42% and 62% of the potential maximum, adjusted for water availability, as determined using the canola simulation model Agricultural Production Systems sIMulator (APSIM; www.yieldgapaustralia.com.au/maps/) which considers soil, water and management practices (Holzworth et al. 2018).

Blackleg crown canker severity (internal infection as reported in Marcroft et al. (2012)) has been measured from cultivars sown at between 30 and 60 sites across the canola-growing regions of Australia each year between 2004 and 2019 (Fig. 1). Nationally, blackleg disease severity has increased from 16.5% average internal infection in 2004 up to a peak of 47.1% in 2010, before slowly declining to 32.2% in 2019. Interestingly, the national average disease severity appears to be decreasing while at the same time canola intensity is increasing, despite the prediction of higher disease due to inoculum load. Possible reasons for this discrepancy are presented in the following sections.

Stubble management and retention

The primary inoculum source for blackleg disease is ascospores produced by sexual reproduction on crop stubble between growing seasons. Cultural practices, such as crop rotation and isolation from infected stubble, are important to reduce the risk of blackleg disease through avoidance of ascospore inoculum. Factors such as tighter rotations and stubble retention have impacted on the efficacy blackleg management strategies.

In the early 2000s in Australia and Canada, a break of 2 or 3 years between canola crops was common practice, allowing stubble to breakdown and ascospore production to diminish (Salisbury et al. 1995; Gladders et al. 2006; Ghanbarnia et al. 2012). However, in Australia the vast majority of ascospores are released in the first year after the canola crop, and therefore tighter canola rotations are viable (Marcroft et al. 2004). Our survey results suggest that these findings may have boosted the confidence of growers to increase the frequency of canola, as rotations have significantly tightened from a break between canola crops of >3 years in 2000 to the more common practice of growing canola in alternate years in 2018 (Fig. 3b). The tighter rotations in Australia are consistent with trends in

Canada whereby canola is grown in alternate years or with no break at all (canola on canola; Zhang and Fernando 2018). Further to this, tightening of rotations to a wheat-canola-wheat system has also partially been driven by limited options for alternative break crops for specific soil types, such as pulses on acid soils.

Research in Australia and Europe showed that isolation of canola crops by >500 m from the previous year's stubble is sufficient to avoid the highest ascospore concentrations and therefore minimise disease (Marcroft et al. 2004; Gossende et al. 2003). An isolation strategy was easily achievable in the early 2000's when canola was grown in wider rotations but with the tightening of rotations, physical separation is less relevant due to the difficulty of isolating the current crop from stubble. Furthermore, this situation is exacerbated by higher inoculum loads due to the overall increase in production area.

There have been significant changes to farming systems during the past two decades in stubble conservation, particularly with the wide uptake of soil conservation tillage practices. By 2016 no-till or zero-till strategies (practices whereby less than 30% of soil is disturbed) represented 75% of the cropping area in Australia, up from only 25% in 2001 (Umbers 2016; Fig. 2). These changes are reflected in the grower survey, whereby most respondents actively reduced stubble loads (by burning or cultivating) in 2000 in contrast to stubble being conserved in 2018 unless there was a specific reason (e.g. pests, disease or weeds), often resulting in stubble left standing (Fig. 3c, d). The changes in stubble conservation are consistent with minimum tillage practices. Machinery with GPS guided systems allows precision seeding of crops in the inter-row between stubble of the previous crop, much of which is left standing and intact after harvest, an innovation that was adopted in the mid-2000s in response to the increase in no-till practices (Fig. 2).

Sowing and flowering dates

Sowing and flowering times of canola crops in Australia have moved earlier which is supported by growers' responses in our survey (Fig. 3e). In 2000, the median sowing date was early-May but by 2018 this had significantly shifted approximately 3 weeks earlier to mid-April. Similarly, targeted flowering times across all regions were an average of approximately 3 weeks earlier in 2018 compared to 2000. Comparison of responses by individuals were analysed for sowing and flowering date from 2000 to 2018, with 38% of respondents sowing 20 days earlier and 47% of respondents with crops flowering 20 days earlier (Fig. 3e).

Timely sowing and flowering is key to optimising canola yield potential. Kirkegaard et al. (2016b) reported declines in grain yield potential (6–6.5%), oil content (0.5–1.5%) and water-use efficiency (3.8–5.5%) for each week delay in sowing after early-April. Timely flowering can increase grain

yield by up to 20% (J. Lilley pers. comm.). The critical period for yield development in canola occurs between 150 and 450 °C days after the start of flowering, with a peak at 350 growing degree days (Kirkegaard et al. 2018). During this phase, canola is unable to recover or compensate for reductions in grain and oil yield caused by stress events. In response to this, optimal start of flowering times using a crop simulation analysis have been identified at a regional level in Australia to avoid the impacts of abiotic stresses such as heat, frost and water stress (Lilley et al. 2019). However, the simulation analysis does not yet account for the effects of biotic stresses such as upper canopy blackleg infection, which are more likely to limit yield of earlier flowering crops (Sprague et al. 2018).

Earlier sowing has been facilitated by the development of improved germplasm and equipment. The introduction of herbicide-tolerant varieties allows in-crop control of weeds, which is a key rotational benefit of canola in predominantly cereal-based farming in Australia (Angus et al. 2015). Precision seeders and satellite-guided sowing with 2 cm accuracy have enabled establishment without cultivation into a seed bed. The previous practice of destroying canola stubble, normally by burning and then raking, leads to disturbing and drying the soil. However, the use of modern sowing equipment maintains the crop residue, minimising moisture loss through soil disturbance, with the seed placed next to the previous season's residue. Additionally, hybrid cultivars with larger seed and more vigorous seedlings are better adapted to sowing in stubble conservation systems. Consequently, the combination of herbicide-tolerant cultivars, satellite-guided sowing equipment and hybrid seeds have resulted in farmers confidently sowing into dry soil that has full stubble retention. Therefore, crops are now often sown by calendar date rather than by soil moisture/weed status and, in some situations, sown dry prior to a rainfall event.

Rainfall is a major driver of maturation of *L. maculans* pseudothecia on stubble and ascospore release (McGee and Emmett 1977; Marcroft et al. 2003). Infection of canola seedlings prior to the 6th leaf stage leads to the development of severe crown cankers (Marcroft et al. 2005). Crops sown in early May remain in this vulnerable growth stage during peak ascospore release in autumn and winter (Fig. 1a) leading to severe crown canker. However, crops sown in early April grow rapidly through the vulnerable stage and likely avoid prolonged exposure to ascospore release, and therefore develop less severe crown canker. These suggestions are consistent with the national disease severity data, whereby blackleg crown canker severity has declined since 2010 (Fig. 2).

Upper canopy infection

While the timing of flowering is not important for development of crown canker, earlier flowering is associated with

increased severity of blackleg disease symptoms on plants post-stem elongation, collectively termed 'upper canopy infection' (Sprague et al. 2018; Fig. 1b). Upper canopy infection (UCI) refers to the development of lesions on the upper stems, branches, flowers, peduncles and siliques of canola. Yield-limiting symptoms were first detected in Australia in 2010 with *L. maculans* identified as the causal agent. Since then, the incidence and prevalence of UCI has increased. In field experiments, reductions in grain yield of up to 30% have been associated with UCI, as estimated by a comparison of crops with complete blackleg control by multiple fungicide applications with an untreated crop (Sprague et al. 2017). In Australia, peak ascospore release from stubble in contact with the ground usually occurs in the winter months from June until August (McGee and Emmett 1977), therefore the earlier flowering time exposes the reproductive parts of the plant to ascospores and increased frequency of cool, wet conditions that favor infection (Fig. 1).

Stubble conservation driven partly by satellite-guided sowing equipment, may also contribute to the emergence of UCI as stubble remains standing and persists for longer because only the root section is in contact with the ground. Compared to stubble knocked down and in contact with the ground, standing stubble is drier with delayed maturation and release of ascospores (McCredden et al. 2018), which may add to the prevalence of upper canopy infection.

Genetic diversity in canola germplasm

blackleg disease is managed through a three-pronged approach: genetic resistance, cultural practices, and fungicides (Van de Wouw et al. 2016; Zhang and Fernando 2018; Fitt et al. 2006). In Australia, genetic resistance is the main control strategy for growers, with two types of resistance: quantitative (minor-gene) and qualitative (major-gene). Quantitative resistance, often considered partial resistance, is thought to be conferred by multiple genes, each contributing a small part to the overall resistance phenotype (Delourme et al. 2006; Stuthmann et al. 2007). The genetics of this quantitative resistance are still poorly understood and only a small number of quantitative trait loci have been identified (Huang et al. 2019; Larkan et al. 2016; Raman et al. 2012, 2016, 2018, 2020). In contrast, qualitative or major gene resistance is considered a complete resistance and is controlled in a gene-for-gene manner, whereby for each resistance gene in the plant there is a corresponding avirulence gene in the pathogen (Flor 1955; Balesdent et al. 2005). For the host side of the *Brassica* spp. - *L. maculans* interaction, a total of 15 major resistance genes have been mapped and two cloned, whilst on the pathogen side 13 avirulence genes have been mapped and 11 have been cloned from 8 loci (Fudal et al. 2007; Gout et al. 2006; Balesdent et al. 2013; Ghanbaria et al. 2012, 2015, 2018;

Parlange et al. 2009; Plissonneau et al. 2016, 2017; Van de Wouw et al. 2009, 2014; Larkan et al. 2013, 2015; Delourme et al. 2004, 2006; Petit-Houdent et al. 2019). Major gene resistance, when effective, protects all parts of the plant (Elliott et al. 2016), however this resistance can be rendered ineffective due to changes in the fungal population.

Resistance breakdown has occurred in Europe (Rouxel et al. 2003; Brun et al. 2010), Canada (Zhang et al. 2016b), and most dramatically in Australia (Sprague et al. 2006b), whereby resistance has been overcome in less than 3 years and caused >90% yield loss. As such, these major resistance genes require management to prevent resistance breakdown. In Australia, Canada and France, all commercially released cultivars are characterised for their complement of known major genes for blackleg resistance (Marcroft et al. 2012; Van de Wouw and Howlett 2020; Zhang and Fernando 2018). This information is used by growers and advisors to rotate cultivars with different resistance genes (Van de Wouw and Howlett 2020). However, the success of resistance gene rotations for disease control is underpinned by knowledge on the structure of the local pathogen population where the genes are deployed and the effect of genes individually and in combination, many of which are complex. For example, *L. maculans* has multiple avirulence genes (*AvrLm1-3*, *AvrLm4-7* and *AvrLm5-9*) that interact with more than one host resistance gene (Parlange et al. 2009; Ghanbarnia et al. 2018; Larkan et al. 2013). In addition, some avirulence genes in *L. maculans* are linked and can be subject to selection even in the absence of the corresponding resistance gene (Van de Wouw et al. 2010). Comparisons between experiments on the epidemiological and evolutionary responses to resistance genes are confounded by multiple factors, such as the genetic background of host lines, which can be further complicated by the possible presence of quantitative blackleg resistance (Brun et al. 2010). Other factors include the unknown structure of the starting pathogen population, lack of markers for avirulence genes - which interact with the resistance genes - and the inability to rapidly screen whole populations for avirulence frequency profiles, which would enable detection of avirulence genes at low frequencies.

In experimental and agricultural systems, *L. maculans* has overcome major gene resistance in as little as 3 years (Sprague et al. 2006a; Rouxel et al. 2003; Brun et al. 2010). However, deployment of resistance genes in space and time can be an effective tool to reduce disease severity and manipulate evolution of pathogen populations (Bousset et al. 2018; Marcroft et al. 2012). In experiments conducted in the field to replicate combinations of spatial and temporal deployment of host resistance genes and dispersal of pathogen populations, the strategic deployment of resistance genes reduced blackleg severity and frequency of virulent isolates, even when resistance genes have been overcome (Bousset et al. 2018). Furthermore, Bousset et al. (2018) reported selection for isolates virulent

towards multiple resistance genes in cultivars with the corresponding set of (stacked) resistance genes, but selection against these isolates in cultivars lacking the corresponding genes. Therefore, for rotation of cultivars to be used to manipulate fungal populations, cultivars with fewer resistance genes provide the most sustainable strategy.

The diversity of major resistance genes present in Australian cultivars has increased over the past 20 years (Fig. 2). Genetic diversity within Australian cultivars released prior to the year 2000 has been traced to 11 ancestral lines, many of which harboured *Rlm4* and/or *Rlm3* (Cowling 2007); this was reflected in our survey, whereby >70% of cultivars grown by respondents in 2000 contained resistance genes *Rlm4* and/or *Rlm3*. Since that time, seven additional resistance genes have been introduced into Australian varieties: *Rlm1*, *Rlm5*, *Rlm6*, *Rlm7*, *RlmS*, *LepR1* and *LepR3* (Fig. 2). Whilst this increase in genetic diversity provides an opportunity to develop strategies to rotate genes to control blackleg, 73% of the cultivars grown in 2020 contain two or more resistance genes, with *Rlm1* and/or *Rlm4* present in all cultivars.

Fungicides for crop protection

Fungicides are an important tool for controlling blackleg in Australia. In the early 2000s, fungicides applied to seed and fertilizer at sowing became commercially available to provide significant control for blackleg disease (Khangura and Barbetti 2002, 2004; Marcroft and Potter 2008). Since 2011, several foliar fungicides have been commercialised for in-crop blackleg control (Van de Wouw et al. 2016; Fig. 2). Prior to the release of these foliar fungicides, all blackleg management decisions were made prior to or at sowing i.e. cultivar, paddock selection, and seed- or fertilizer-amended fungicides.

Since 2000, there has been a significant increase in fungicides applied at sowing (seed and/or fertilizer) to control blackleg in Australia (Fig. 3f). In 2000, 52% of respondents used at least one fungicide applied to seed (fluquinconazole) and/or fertilizer (flutriafol), 28% did not use any fungicide and 20% applied fungicides only when necessary. In 2018, 95% of respondents always used at least one of these fungicides, with the remainder using a fungicide if necessary. Of the respondents that always used a fungicide, those using only a seed dressing declined from 40% to 22% and the use of both fungicides increased from 2% to 55%. The significant increase in fungicide use was consistent across rainfall zones and states. As mentioned earlier, crown canker severity may be lower in earlier sown crops, which grow rapidly in warmer conditions and avoid ascospore showers during vulnerable stages of plant development. Therefore, fungicide application at sowing may not be warranted in some seasons or regions with blackleg controlled by strategic applications of foliar fungicides instead. The level of foliar fungicide use was captured in the

survey for 2018. Of the respondents 49% applied a foliar fungicide if they perceived it was required dependent on seasonal conditions, the level of cultivar resistance and disease pressure, with the remainder evenly split between always (20%) and never (23%) applying a foliar fungicide.

Blackleg upper canopy infection on plants has become more prevalent in Australia since 2011 (Sprague et al. 2018). Research indicates that foliar fungicides applied during early lowering to control *Sclerotinia* stem rot (caused by *Sclerotinia sclerotiorum*) is also effective against *L. maculans* (Sprague et al. 2017). There are currently no registered products for control of blackleg upper canopy infection, however, some products registered for *S. sclerotiorum* control are effective against *L. maculans*. The majority of survey respondents (61%) considered fungicide application to control upper canopy infection if one was available, likely a reflection of the increase in prevalence and potential for high yield losses due to upper canopy infection (Sprague et al. 2018). Respondents were asked whether blackleg upper canopy infection was a consideration in the decision to apply fungicides for *Sclerotinia* stem rot. Of the 61 respondents that answered, the majority considered upper canopy infection only if conditions were suitable (52%), some always applied a fungicide specifically for upper canopy infection (14%) and the remainder never considered fungicide control for upper canopy infection (33%). The expansion of fungicide availability for blackleg and *Sclerotinia* stem rot control could result in up to five fungicide applications within a single season (Fig. 1).

Reliance on fungicides for blackleg control is unique to Australia, with little use in Canada and Europe due to inconsistent yield responses (Peng et al. 2020). The increased availability of fungicides and decreasing prices is driving a push towards a reliance on chemicals and genetic resistance, rather than cultural practices for blackleg control. As such, the three-pronged approach has become two, with reliance on the efficacy of these fungicides to minimise blackleg disease. Until recently, all fungicides to control blackleg in Australia were demethylation inhibitors of the ergosterol synthesis pathway (DMI class), resulting in strong selection pressure for the evolution of resistance. Resistance to DMI fungicides in *L. maculans* has been identified in Australia, the first report worldwide (Van de Wouw et al. 2017). Resistance is conferred by multiple mechanisms, such as insertions of fragments of transposable elements into the promoter region of the *Erg11* gene (also known as *Cyp51* in other fungal species; Yang et al. 2020). Fungicide resistance has been detected in approximately 18% of *L. maculans* populations screened from stubble samples across Australia, but the proportion of resistant isolates within these populations and the on-farm implications of fungicide efficacy remains unknown (Van de Wouw et al. 2017; Yang et al. 2020).

More recently, antifungals in the succinate dehydrogenase inhibitor (SDHI) class became available for blackleg control,

either as single modes or a mixture with DMI's, for application to seed or in-crop during vegetative growth, with more effective control than DMI's (Marcroft, unpublished data). The SDHI fungicides are at medium-high risk for the development of fungicide resistance as they act on a single enzyme site (www.frac.info accessed 21 May 2020). Reduced sensitivity to SDHI's are reported in field populations of other fungi, such as *Botrytis cinerea* (e.g. Veloukas et al. 2011), *Zymoseptoria tritici* (e.g. Dooley et al. 2016) and *Alternaria* species (e.g. Avenot et al. 2009), with mutations providing cross-resistance to varying degrees between different SDHI's. Current Australian stewardship guidelines for SDHI's in canola recommend a limit of two applications during vegetative growth for blackleg and two applications during the flowering period for *Sclerotinia* stem rot (www.croplife.org.au, accessed 21 May 2020) despite the fact that *L. maculans* populations will be exposed to sprays applied during flowering.

With the heavy reliance on fungicides to maintain current canola intensity and the potential for up to five fungicide applications within a growing season, strategic use of fungicides to minimise the risk of fungicide resistance is essential. This might include avoiding an application at sowing if sown early and therefore likely to avoid spore release, or the rotation of fungicides with different actives or mixtures to reduce selection pressure. However, most commercially purchased seed in Australia is sold pre-treated with a fungicide, therefore growers may not have the choice to skip a seedling fungicide application.

Research priorities for long-term management of blackleg

From this review of the changes in farming practices, we have identified areas of research that are most likely required to support Australian canola production now and into the future to limit blackleg disease. These include:

- (i) *Developing a framework to implement successful rotation of major resistance genes, to which breeders and industry can then respond with a suitable suite of cultivars and deployment strategies*

While rotation of resistance genes has been shown to minimize blackleg disease (Marcroft et al. 2012; Bousset et al. 2018), there is limited information on appropriate implementation strategies to maximise the benefits for disease control and limit evolution of the pathogen. Recent studies have indicated that not all resistance genes can be rotated in the same sequence (Van de Wouw and Howlett 2020) and that stacking of resistance genes can undermine rotation (Bousset et al. 2018). The frequency of virulence alleles in a population

needed to overcome resistance is unknown. Understanding these gaps in knowledge is essential to develop and implement effective and sustainable use of major gene resistance.

- (ii) *Understanding the impact of earlier sowing times on exposure to spore showers and subsequent development of crown canker*

As identified in the survey, there has been a shift to earlier sowing dates. Earlier sowing potentially allows seedlings to germinate and grow quickly through the vulnerable stage of development that leads to crown cankers (Marcroft et al. 2005). Therefore, earlier sowing may potentially reduce the need for fungicides at the seedling or 4–10 leaf stages. Further investigation is required to enable recommendations to industry on the use of fungicide application at sowing.

- (iii) *Screening for genetic resistance (major gene and quantitative) to upper canopy infection*

The prevalence of upper canopy infection has increased rapidly due to changes in flowering times and potentially exacerbated by changes in stubble management. Preliminary data suggest that effective major gene resistance can control upper canopy infection (Sprague et al. 2018), whilst the contribution of quantitative resistance is unknown. The development of a phenotyping method to screen upper canopy infection is required to allow selection of resistant genoplasm.

- (iv) *Assessment of novel strategies to reduce inoculum carryover on stubble in minimum tillage systems and under predicted increases in canola production and (v) identifying the epidemiology of stubble retention such as timing and quantity of ascospore release*

Preliminary data suggest that the maintenance of stubble in the farming system has significantly changed the patterns of ascospore release, potentially leading to higher inoculum levels later in the growing season and in the following season. Further research is needed to determine the full extent of this change e.g. is ascospore release greater in the second year when standing stubble is knocked down, therefore potentially increasing disease risk? These potential changes in disease epidemiology are not specific to blackleg disease but are relevant to other stubble borne disease.

- (v) *Monitoring and predicting the evolution of fungicide resistance*

Increased fungicide use increases the risk of the evolution of fungicide resistance. Fungicide resistance has already been detected to the DMI fungicides (Van de Wouw and Howlett 2012; Yang et al. 2020), but the levels of this resistance in the

field and the implication for their efficacy in the field is still being investigated. Monitoring for resistance towards the new fungicide classes as well as the development of fungicide resistant management strategies should be a priority as these fungicides gain momentum.

- (vi) *Developing genetic tools for high throughput analysis of blackleg populations to improve understanding of evolutionary drivers for resistance genes and fungicides.*

Analysis of genes involved in virulence and fungicide resistance has shown that the genes are found in specific AT-rich repetitive regions of the fungal genome (Rouxel et al. 2011). As a consequence of the gene location, these genes are readily mutated due to the movement of transposable elements. Understanding genetic structures and identification of these mobile genetic elements may be useful in understanding which populations are at risk of evolving virulence/fungicide resistance and allow the development of molecular markers for tracking such changes in population.

Conclusions

There has been a rapid shift in farm management practices in Australia as growers adapt to changes in climate and technology, availability of improved genetics, and advances in weed and pest control. The intensification of production, conservation tillage practices, and earlier sowing and flowering times have significant implications for the epidemiology and integrated management of blackleg disease (summarised in Fig. 1b).

Although this review is of the changing farming systems in Australia, the findings are of international significance given the global importance of blackleg and consumer expectation to improve sustainability of canola production, particularly in regard to chemical pest control. In this context, many of the identified research foci are of broader relevance to all canola-producing regions of the world, particularly the areas of resistance gene management and characterisation of pathogen populations.

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