Long-term analysis of Australian canola variety resistance ratings 2002 - 2008

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ABSTRACT

We analysed the complete set of data available from 2002 to 2008 from national blackleg disease trials in order to assess temporal changes in resistance in canola (*Brassica napus*) varieties to blackleg disease. The basic disease measurement (blackleg disease score) on plots in these replicated trials was percentage survival, determined by dividing the number of plants surviving at maturity by the number of established seedlings. Square-root transformed disease scores on an individual plot basis were analysed using spatial analysis within sites and multi-environment trial analysis with a factor analytic (FA) mixed model framework across sites. Data were analysed in blocks of 3, 4, 5, 6 and 7 years, to compare the disease ratings from long-term analysis with the current system of restricting data to the previous 3 years. In the 7-year analysis, for varieties moderately resistant or less, there was no evidence for a change in disease score over years. Varieties could be classified into two groups – varieties that showed no change in disease score when plotted against FA1 loading, and those that decreased in disease score as FA1 increased. FA1 loading is interpreted in this case as site mean disease level. These results will influence the future assessment and interpretation of variety resistance ratings published annually in Australia.

Key words: Blackleg disease – resistance – spatial analysis - multi-environment trial analysis – factor analytic models

INTRODUCTION

Blackleg disease of canola (*Brassica napus* L.), caused by *Leptoshpaeria maculans*, is one of the most economically devastating diseases of canola in Australia and world wide (West et al. 2001) causing seedling death, crop lodging and early senescence. Blackleg disease is often associated with losses in grain yield ranging between 10% and 50% (West et al. 2001). Resistance reduces the impact of blackleg disease in Australian farming systems, along with cultural methods of removal of infected stubble by burning or tillage, and long rotations or geographic separation from fields containing diseased stubble. Blackleg resistance has increased in Australia over 30 years 1970 – 2000 as a result of breeding efforts (Cowling 2007). Blackleg, however, remains a major threat to future production of canola Australia-wide and this threat is exacerbated by increasing acreage planted to canola.

The pathogen undergoes sexual recombination at the beginning of every season on the stubble of host plants. This ensures large levels of genetic recombination, followed by large-scale ascosporic dispersal. Selection pressure is imposed by host resistance. This has resulted in breakdown of resistance in such varieties as Surpass 400 with a major gene for resistance (Li and Cowling 2003), and has been reported to "erode" polygenic resistance (Cowling 2007). Salisbury et al. (1995) also reported an erosion in field (polygenic) resistance to blackleg in Australian canola varieties after a few years of cultivation, with some varieties facing a gradual erosion of resistance (e.g. Maluka, Eureka) and other cultivars (such as Yickadee) undergoing a much more rapid rate of erosion. In Australia, strains of *L. maculans* are present that can overcome major genes for resistance due to rapid selection for specific strains of *L. maculans* (Sosnowski et al. 2001). Further, Australia has one of the highest diversity of isolates of *L. maculans* (Balesdent et al. 2005).

Blackleg resistance ratings for Australian canola varieties are published annually by the Canola Association of Australia (CAA) with support from the Grains Research and Development Corporation, and the trials are carried out by public and private canola breeding programs and co-ordinated by a blackleg committee. There are sometimes large changes in published ratings

for a variety from year to year, which could be due to chance, biological changes in resistance in the field or an artefact of the analysis. There is some evidence from historical published ratings to suggest that Australian canola varieties decrease in resistance ratings at an average of 0.15 resistance units per year (Cowling 2007). However these variations are difficult to explain based solely on biological changes, and there is a need to critically assess the current analysis procedure. Further, there are many reports of large genotype by environment effects, which could be due to climatic influences on disease development, or from the variation introduced by stubble source across disease nursery sites. This project aims to re-evaluate these apparent changes in varietal resistance over time based on the analysis of the entire data set of the NBG from 2002 to 2008. This paper will use the entire data set from 2002 to 2008 to test the following hypotheses:

- **i.** That the inclusion of more years of data in the analysis will demonstrate that genotype by environment interactions for disease ratings vary greatly from year to year and site to site, and therefore more years of data will lead to more stable assessment of disease ratings.
- **ii.** That for some varieties there is no change in average disease ratings over years.
- **iii.** That a minimum number of years, sites and varieties are necessary in trials in order to detect which varieties show a significant "change in resistance" from year to year.

METHODS AND MATERIALS

Disease Nurseries

The data set for this study consisted of commercial and unreleased varieties grown in disease nurseries under field conditions during the years 2002 to 2008. Such nurseries are designed and run by government and commercial breeding companies such as, The Department of Primary Industries Victoria, New South Wales Department of Primary Industries, South Australian Research and Development Institute, Department of Agriculture Western Australia, Nuseed, Bayer CropScience, Pacific Seeds, Pioneer Hi-Bred and Canola Breeders Western Australia Pty Ltd. Disease nurseries were established on canola stubble from the previous season. Trials were designed as incomplete block row-column designs, neighbour balanced designs and randomised complete block designs, with replicated varieties. Disease nurseries were located in high-medium rainfall regions of WA, SA, VIC and NSW. The process of conducting trials and publishing disease resistance ratings is managed by a "blackleg review committee" (BRC) composed of representatives of the above organisations.

Disease Assessments

The basis of disease assessment was percentage plant survival, carried out by counting all seedlings after emergence at the open cotyledon stage but before seedling death, and then recounting the total number of plants at plant maturity, at the windrowing stage. This method of disease assessment has been used in all canola breeding programs in Australia for the past 30 years. It is readily applied to a large number of field trials by different operators, thereby increasing exposure of cultivars to a diverse array of blackleg isolates. Further, this measure also reflects the economic cost of blackleg on cultivar yield potential. Disease nurseries were monitored to ensure that plant death from factors other than *L. maculans* were kept to a minimum and no other disease or insect burden had the potential to cause damage. Plots with less than 20% emergence were defined as missing for the analysis. A minimum disease level on control susceptible cultivars was also required for a trial to be included in the analysis. The raw data of percentage stand decline was then transformed by square root, forming the basis for disease resistance modelling and analysis.

MET analysis and FA models

Data sets were modeled in groups of 3 to 7 years and analyzed according to the methods developed and described in Smith et al. (2001) for large unbalanced data sets, that is, not all varieties were present in all trials, according to the current procedures of testing by the BRC. Complete mixed model analysis was carried out using the Factor Analytical (FA) model structure with fixed experiment effects and random variety x site effects. A maximum of two factors were used in all analyses. After modeling, BLUPS were used in the prediction of variety effects over sites and years (Smith et al. 2001). Loadings scores for the first and second factors from the FA model were produced graphically as biplots to determine site by year correlations.

All statistical analyses were carried out using ASReml - R package (Butler et al. 2007).

RESULTS

Varieties x Years x Sites

Predicted values of disease scores for varieties showed little if any trend over the sites and years in which they were tested (Fig. 1). Moderately resistant varieties such as AgCastle and AvSapphire showed less variation across sites and years than susceptible varieties such as Karoo, ATR-Stubby and Q2, which varied significantly across sites. For example, mean disease ratings on Karoo ranged from 5.5 to 0 across sites and years.

Variation within Disease Nurseries

Biplots showed variation in ranking of genotypes in trials at a site between and within years, and variation in the proportion of variance accounted for by genetic variation among varieties (Fig. 2). For example, some trials at Laharum, VIC, had relatively high genetic correlations to each other; and others had poor or no genetic correlation (perpendicular vectors). The proportion of variance accounted for by genetic differences between lines ranged from 53% to 89% at Laharum.

Some trials at Mt Barker, WA, were poorly correlated with one another, and others were highly correlated (vectors close together).

Most trials at Marrar, NSW, had a high proportion of variance accounted for by the model.

Trials at Moyhall, SA, appeared to be clustered over years, although the proportion of variance accounted for by the model ranged widely from 39.3% to 100%.

Changes in Varietal Resistance against Site Mean Disease Level

Selected varieties were plotted against site loadings for FA1, which represented the site mean disease level (Fig. 3). This was carried out for the 2002 – 2008 data set. Varieties were separated broadly into two groups – those that were more or less stable in disease score as mean site disease level increased (typified by AV-Sapphire), and those that had significant negative slopes as mean site disease level increased (such as ATR-Beacon, ATR-Stubby, Q2, Karoo and Mystic).

DISCUSSION

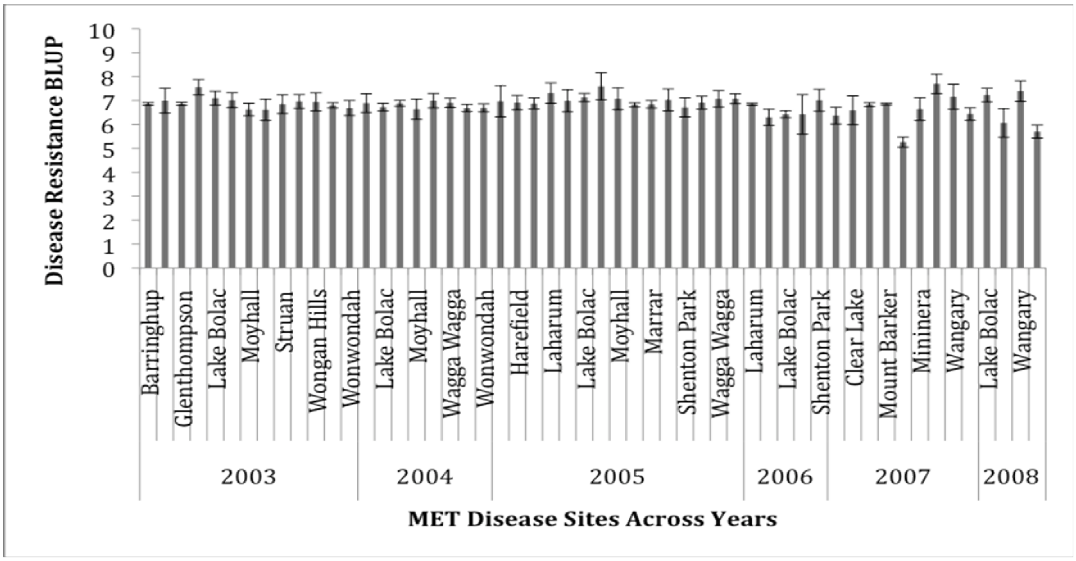
In this assessment of the national blackleg ratings for Australian canola varieties, it is apparent that it is difficult to predict the ranking of varieties at any particular site in any particular year. Sometimes large changes occur for specific varieties from year to year, but there are few consistent changes across years. Cowling (2007) and Salisbury et al. (1995) claimed that there was an erosion of resistance of specific varieties over years. The former study reported that Australian canola varieties decrease in resistance rating at an average of 0.15 resistance units per year based on the annual CAA publication of variety resistance ratings. However the annually-published blackleg ratings use only the previous three years of data for the current year publication. There was no consistent “erosion” of resistance across the years 2002 to 2008 for varieties in this analysis.

A significant result of this analysis was the slope of varietal resistance plotted against the loadings of FA1 from the factor analytic model (Fig. 3), which showed how predicted values for varieties reacted as site mean disease levels increased. There were three possible slope responses: increasing, decreasing or constant disease scores across increasing site disease levels. The majority of varieties were either stable in disease score across site mean disease levels (e.g. AV-Sapphire) or decreased in disease score (more disease) as site mean disease increased (e.g. Mystic) (Fig. 3).

The results showed that Warrior CL declined in resistance as the mean level of disease at nursery sites increased. Under high disease pressure, this variety appeared more susceptible to disease. The CAA published disease ratings for Warrior CL began with a rating of R in 2007 followed by a drop to MR in 2008 and a recovery to MR-MS in 2009. There was a similar picture among the TT varieties, ATR-Stubby and CB Trigold, all of which had large decline in disease scores (more disease) as site mean disease increased. These varieties also showed large variation in resistance ratings from site to site across the 2002 – 2008 data analysis. The published disease ratings of Ag-Spectrum changed from MR in 2007 to MS in 2008, then to MR-MS in 2009. However, Ag-Spectrum had relatively stable disease ratings from site to site over years in this study, and there was no trend towards greater susceptibility

over years. There is an indication that the three-year analysis system currently used in the CAA publication is influenced greatly by the particular data sets available within a particular 3-year period, and that a longer analysis period may be justified.

(a) AV-Sapphire



(b) Karoo

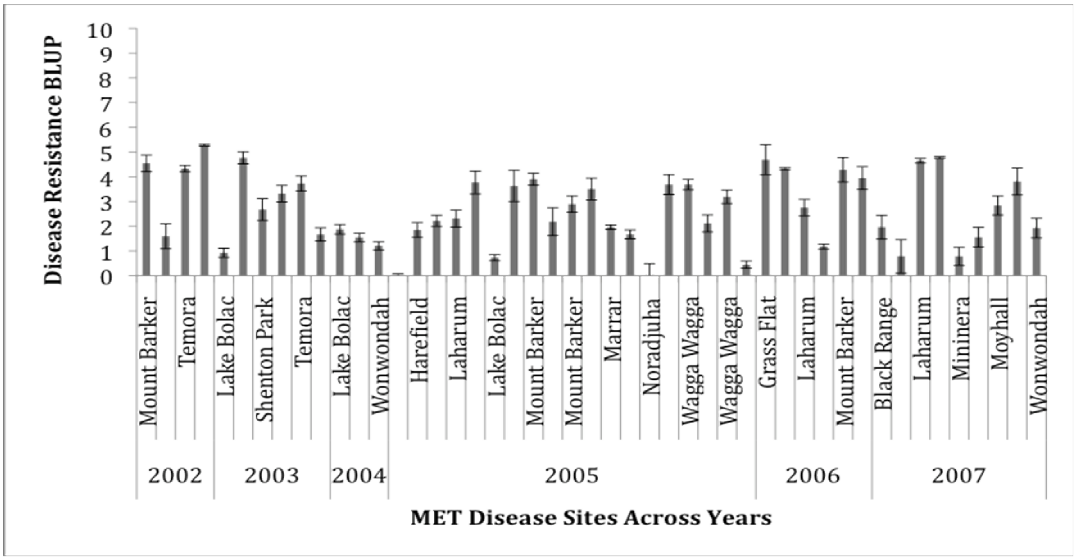


Fig. 1. Predicted values (BLUPS) across sites for (a) AV-Sapphire and (b) Karoo plotted across sites and years for the 2002 – 2008 data set.

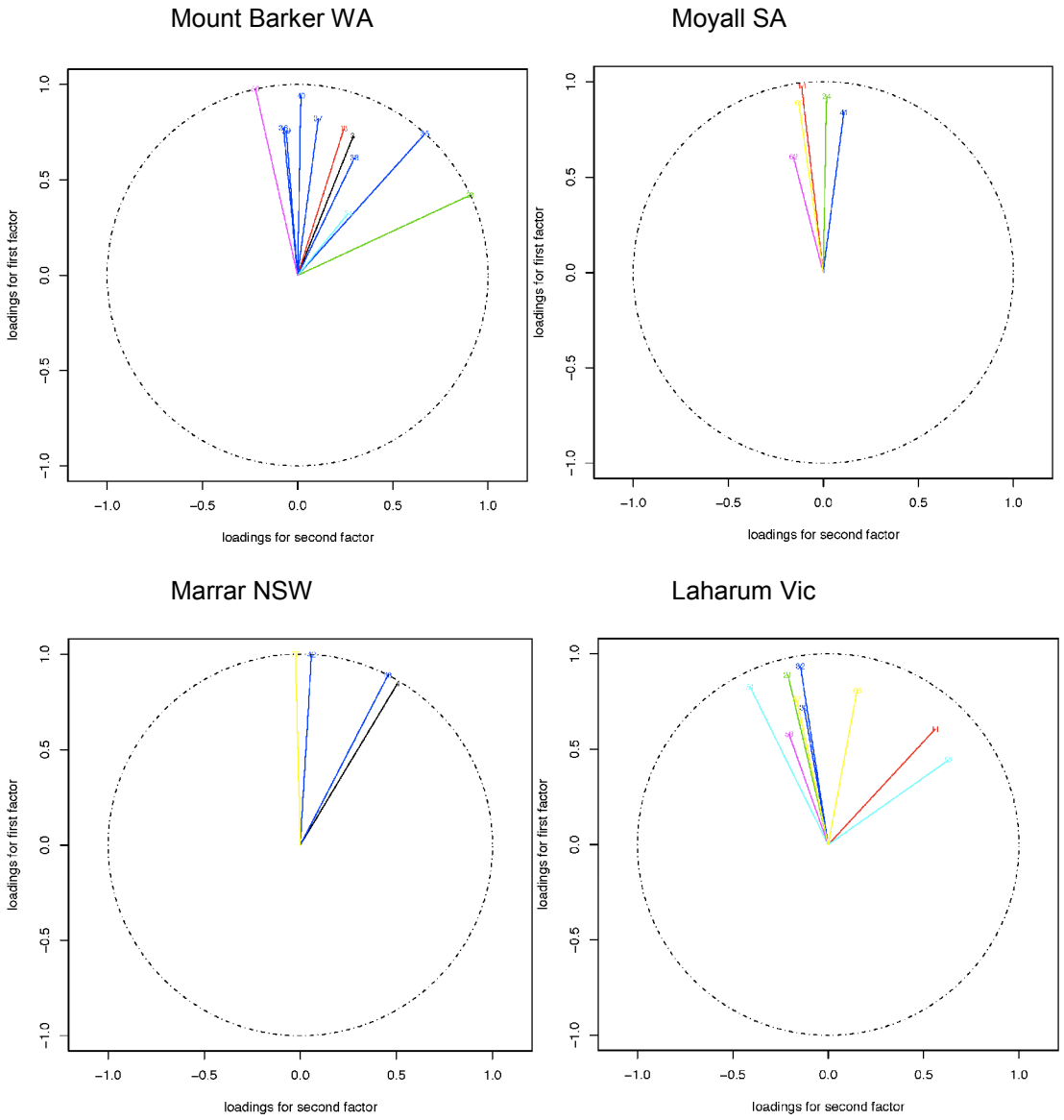
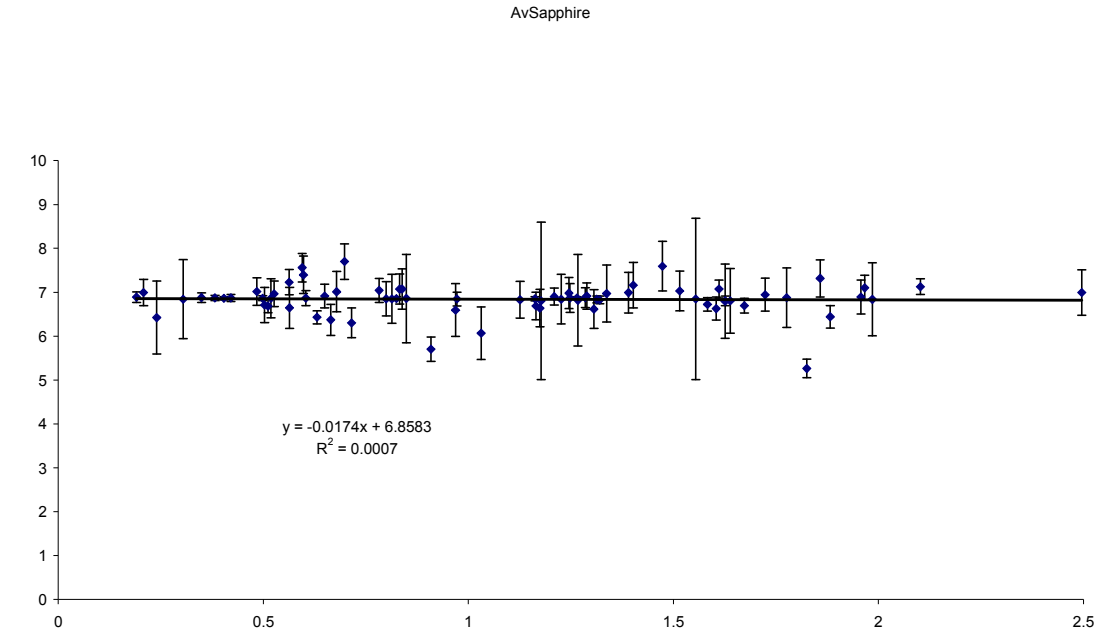


Fig. 2. Biplots of selected blackleg disease nursery trials Mount Barker WA, Moyhall SA, Marrar NSW and Laharum VIC during the years 2002 – 2008. Sites are colour coded according to year (2002 = black, 2003 = red, 2004 = green, 2005 = dark blue, 2006 = light blue, 2007 = pink and 2008 = yellow).

Disease scores on Av-Sapphire were independent of site disease resistance levels (Fig. 3). However, published ratings of Av-Sapphire decreased from MR in 2007 to MR-MS in 2008 and 2009. If it were possible to detect this "stable" pattern of behaviour in the first or second year of testing, it might be possible to predict more "stable" resistance ratings than at present.
In a study by Smith et al. (2005) on variety yield, it was noted that if the primary goal of the analysis was to provide an accurate estimation to growers of varietal performance across diverse environments, the number of years to sufficiently provide a representative sample of seasons should be between 5 – 10 years of data. A similar conclusion may be reached from the national canola blackleg resistance ratings. However, most varieties are obsolete well before this time; therefore a "predictor" of variety behaviour is important. The plot of variety

disease score vs FA1 loading may be a valuable predictor of future variety performance for blackleg resistance (Fig. 3). Another valuable predictor may be the level of variation in disease score from site to site (Fig. 1). Susceptible varieties appear to have more variation between sites, and a higher negative slope vs FA1, than resistant varieties. Varieties with strong negative slopes vs FA1 may be be flagged with additional warnings. Such varieties may be detected earlier by including more years in the data analysis.

(a) AV-Sapphire



(b) Mystic

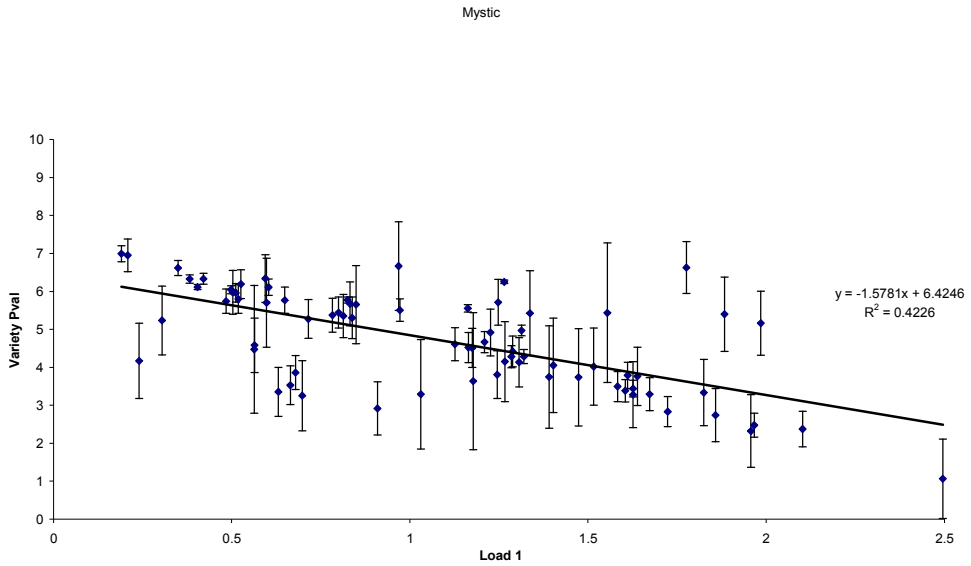


Fig. 3. Varietal BLUPS plotted against site FA(1) Loadings for the NBG 2002 – 2008 data set.

Genetic correlations between sites is utilized by the FA model to 'borrow' information for varieties that are missing in trials, which is a key advantage of using FA mixed model analysis. It is important to understand the causes of low correlation between sites. Weather factors such as rain, wind and temperature have been demonstrated as having an impact on all stages of the disease life cycle, with particular importance to ascospore and pycnidiospore production (Howlett et al. 2001). Stubble source will probably also be important in varietal resistance ranking, through its influence the genetic structure of the fungal population. Li et al. (2008) reported that environmental differences between blackleg nursery sites in Australia could have an impact on the level of expression of host resistance, which was also demonstrated by previous studies (Sosnowski et al. 2001). Thus environmental factors can have an impact on disease incidence and severity across locations, and are a potential source of G x E. In future research, such factors will be analysed for their impact on varietal disease score.

In NSW DPI disease nurseries, the stubble is created every year from a standard mix of polygenic and major gene varieties (pers. Communication Neil Wratten NSW DPI). However in SA and WA the stubble source is the variety grown in the farmers paddock before the disease nursery trials (pers. Communication Neil Wratten NSW DPI). This variability in stubble source across sites could contribute to the G x E variations across environments as it is the medium on which genetic recombination of the fungus occurs, and has the potential to produce patho-types capable of attacking important sources of genetic resistance. Stubble source is currently unaccounted in the analysis procedures and may have different rates of impact on varietal survival across nursery sites.

Disease scores in trials at Mount Barker in WA were often poorly correlated (Fig. 2). The management practices at Mount Barker could possibly be an explanation. Within 2005, varieties were planted in separate disease nurseries according to the variety type: conventional, TT or Clearfield System. All disease nurseries had the variety Pinnacle as the stubble source. The genotypes in each trial were mostly different with few common genotypes, and this most likely explains the lack of correlation between Mount Barker sites within 2005. However, trials in the years 2004 and 2007 were not correlated with each other and this may be due to stubble source or genotypic variation.

Marcroft et al. (2004) found that the type of cultivar stubble had an impact on pseuthothecial densities and hence the number of ascospores produced, which can have varying effects across different nursery sites. The recent study by Li et al. (2008) found that among the blackleg disease nursery sites across Australia, there were significant differences between VIC, SA and WA but not in NSW. They also found variations in rankings of cultivars across sites. This is further evidence of G x E variation introduced by stubble source at disease nursery sites across Australia, which may impact the blackleg disease ratings.

This study suggests that rolling 3-year data sets do not contain sufficient data to accurately predict varietal blackleg disease ratings in canola in southern Australia. This could explain the fluctuation in CAA published variety ratings from year to year. By using the entire data set 2002 - 2008, with many varieties in common across years, we found little evidence for "erosion" of resistance in polygenic varieties. There was a significant amount of G x E interaction across disease nurseries which could be explained by low rates of common genotypes between trials – we recommend 15-20% "common" genotypes between trials. Another source of G x E is stubble source. This study could be extended to include data on stubble source, latitude, longitude and rainfall to account for the observed G x E.

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