

## The development of a bivariate mixed model approach for the analysis of plant survival data

Aanandini Ganesalingam<sup>1</sup>, Alison Smith<sup>2</sup>, Cameron Beeck<sup>1,4</sup>, Wallace Cowling<sup>3,4</sup> and Brian Cullis<sup>5</sup>

<sup>1</sup> School of Plant Biology, The University of Western Australia, 35 Stirling Highway, Crawley WA 6009, Australia

<sup>2</sup> School of Mathematics and Applied Statistics, Faculty of Informatics, University of Wollongong, Australia. Email: [alismith@uow.edu.au](mailto:alismith@uow.edu.au)

<sup>3</sup> The UWA Institute of Agriculture, The University of Western Australia, 35 Stirling Highway, Crawley WA 6009, Australia

<sup>4</sup> Canola Breeders Western Australia, 15/219 Canning Highway, South Perth WA 6151

<sup>5</sup> School of Mathematics and Applied Statistics, Faculty of Informatics, University of Wollongong, Wollongong NSW 2522, CSIRO, Australia  
E-mail: [ganesa01@student.uwa.edu.au](mailto:ganesa01@student.uwa.edu.au)

### ABSTRACT

The Canola Association of Australia's blackleg resistance rating for commercial canola cultivars uses plant survival as a measure of disease resistance. Plant survival is assessed by taking plant counts at emergence and then at maturity; that is before and after disease infection. These counts are then used to form a derived variable: plant counts at maturity expressed as a percentage of plant counts at emergence, which is then subject to mixed model analysis. Our study instead proposes a bivariate linear mixed model approach, where the two counts are treated as two 'traits', which are log transformed before analysis. We demonstrate this approach to the analysis of the nine blackleg disease resistance trials from the 2009 growing season. From the bivariate model, Best Linear Unbiased Predictors (BLUPs) of variety effects at the two sampling times were used to produce two plots, which represented three possible indices for selection: counts at emergence, counts at maturity and percentage survival score. In this paper, we discuss these plots for the two disease nurseries; Baker's Hill and Bordertown. The bivariate mixed model approach to the analysis of blackleg disease resistance provided a more detailed 'picture' of the impact of disease resistance in comparison to using a single derived variable from these two traits.

**Key words:** Blackleg disease – resistance – bivariate mixed models

### INTRODUCTION

Blackleg disease of canola (*Brassica napus* L.), caused by *Leptosphaeria maculans*, is one of the most economically devastating diseases of canola in Australia and worldwide (West et al., 2001). Varietal resistance in commercial cultivars is one of the main methods of reducing the impact of blackleg on Australia farming systems (Balesdent et al., 2001). The blackleg resistance ratings in Australia for canola cultivars are published annually by the Canola Association of Australia with disease nurseries run by public and private plant breeding programs coordinated by the National Blackleg Group. Disease resistance is measured by counting the number of seedlings after emergence and then recounting the total number of plants at maturity for each plot. The protocol has been to compute, for each plot, a derived variable, namely the counts at maturity expressed as a percentage of the counts at emergence, called 'percent survival'. This variable is then subject to a transformation before mixed model analysis. We will refer to this method as the historical approach. We instead propose a bivariate mixed model analysis in which the two traits are the counts taken at emergence and at maturity. These counts are then subject to log transformation before a bivariate linear mixed model analysis. The aim of this analysis is to demonstrate that the bivariate model is preferred for the analysis of plant survival data.

## MATERIALS AND METHODS

In this paper we consider two of the nine blackleg nurseries from 2009, namely Baker's Hill (WA) and Bordertown (SA). The number of varieties tested in each nursery was 57 and 74 respectively. At Bordertown varieties were grown in sub-trials depending on their chemistry type. Full details of the data and statistical methods are given in Ganesalingam et al. (2011).

## RESULTS

For the disease nurseries at Baker's Hill and Bordertown, BLUPs of variety means at the two sampling times, emergence and maturity were used to produce two plots. The first was of BLUPs of variety means at emergence plotted against BLUPs of variety means at maturity, Figs. 1 & 3. The second was of the difference between BLUPs of variety means at emergence and maturity (note this corresponds to the percent survival scale) plotted against BLUPs of variety means at emergence, Figs. 2 & 4.

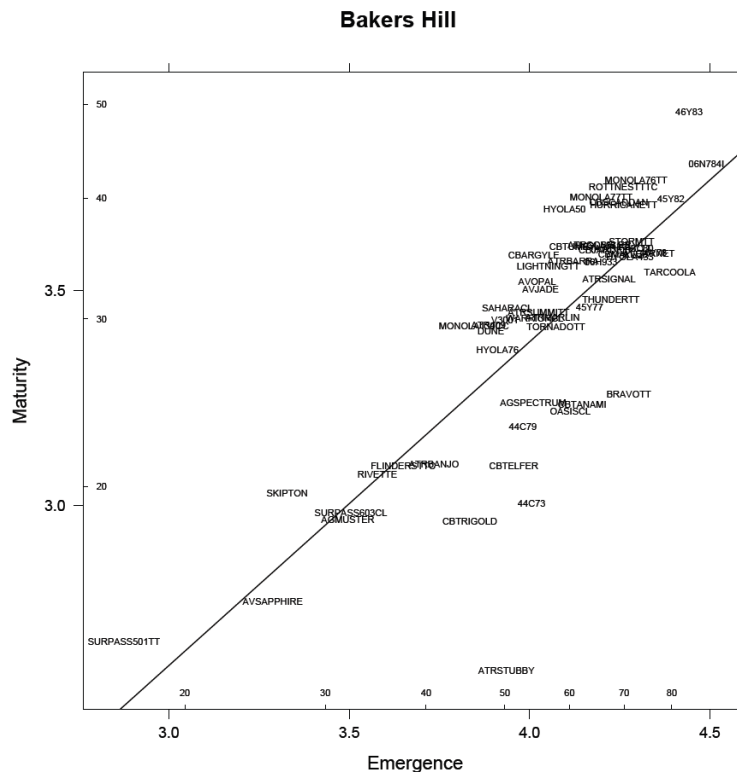


Fig. 1. Predicted variety means at emergence plotted against predicted variety means at maturity from the bivariate model for the disease nursery at Baker's Hill. The axes are on a log scale with the back-transformed scale (i.e. plant counts) shown inside each axis. The regression line represents the regression of maturity against emergence counts.

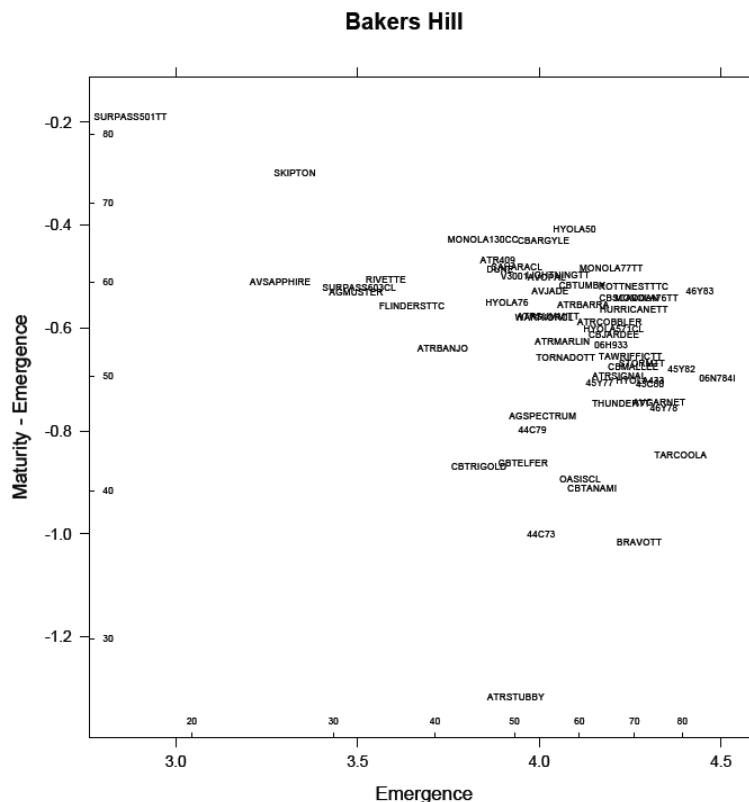


Fig. 2. The difference between predicted variety maturity and emergence means (corresponds to percentage survival when back transformed, these values are shown on the inside of the axes) plotted against predicted variety means at emergence from the bivariate model for the disease nursery at Baker's Hill.

## DISCUSSION

The bivariate model enables an observation of the genetic effects of each trait at a disease nursery. In Figs. 1 & 3 it can be seen that there is genetic variation for emergence at the Baker's Hill and Bordertown disease nurseries. At Baker's Hill, emergence counts ranged from 15 to 90 counts and at Bordertown the emergence counts ranged from 10 to 60 counts. While such variation for emergence is known to be an issue at disease nurseries, the bivariate approach has for the first time enabled this to be quantified. Variation for emergence could arise from seed source variation (Ellis and Roberts, 1980; Forcella et al., 2000) or from the impact of blackleg disease on seedling emergence (Li et al., 2007; Sosnowski et al., 2001). A bivariate approach enables an insight into trait based variation, which would not have been observed under the historical method of analysis.

The regression line of maturity on emergence (implicit in the bivariate analysis) was indicated on each of the plots. The slope of the regression line was 0.62 for Bordertown and 0.75 for Baker's Hill. Both these slope values demonstrate a strong linear relationship between traits despite the fact that the genetic variation in the two traits may arise from different causes.

The relationship between emergence and percentage survival scores of varieties can be seen in Figs. 2 & 4. These plots often show a re-ranking of varieties compared to the plots of maturity counts against emergence counts. For example, at the Baker's Hill disease nursery the plot of percentage survival scores against emergence (Fig. 2) shows that variety 46Y83 had an average percent survival value of only 60%, however in the plot of maturity against emergence counts (Fig. 1), 46Y83 had the highest emergence and maturity counts at the disease nursery. These plots of variety BLUPs at the two sampling times thus provides 3 indices of selection against blackleg disease resistance, namely emergence counts, maturity counts and percentage survival scores. It is then up to the breeder's discretion which disease resistance index (or indices) to select by.



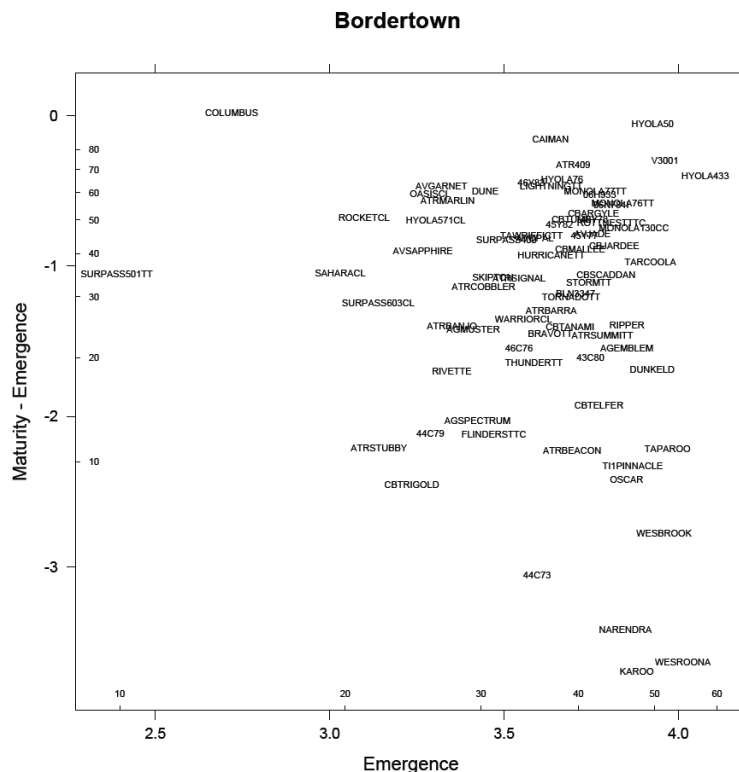


Fig. 4. The difference between predicted variety maturity and emergence means (corresponds to percentage survival when back transformed, these values are shown on the inside of the axes) plotted against predicted variety means at emergence from the bivariate model for the disease nursery at Bordertown.

#### ACKNOWLEDGEMENTS

The authors would like to thank the National Blackleg Committee for the use of the 2009 blackleg data set.

#### REFERENCES

- Balesdent, M.H., A. Attard, D. Ansan-Melayah, R. Delourme, M. Renard, and T. Rouxel, 2001: Genetic control and host range of avirulence toward *Brassica napus* cultivars Quinta and Jet Neuf in *Leptosphaeria maculans*. *Phytopathology* 91, 70-76.
- Ellis, R., and E. Roberts, 1980: Improved equations for the prediction of seed longevity. *Ann Bot-London* 45, 13.
- Forcella, F., R.L. Benech Arnold, R. Sanchez, and C.M. Ghera, 2000: Modeling seedling emergence\*. *Field Crops Res* 67, 123-139.
- Ganesalingam, A., A. B. Smith,, C. Beek, W. A. Cowling, and, B. R. Cullis, 2011: A bivariate mixed model approach for the analysis of plant survival data. (submitted to *Crop and Pasture Science*)
- Li, H., K. Sivasithamparam, and M.J. Barbetti, 2007: Soilborne ascospores and pycnidiospores of *Leptosphaeria maculans* can contribute significantly to blackleg disease epidemiology in oilseed rape (*Brassica napus*) in Western Australia. *Australas Plant Path* 36, 439-444.
- Sosnowski, M.R., E.S. Scott, and M.D. Ramsey, 2001: Pathogenic variation of South Australian isolates of *Leptosphaeria maculans* and interactions with cultivars of canola (*Brassica napus*). *Australas Plant Path* 30, 45-51.
- West, J.S., P.D. Kharbanda, M.J. Barbetti, and B.D.L. Fitt, 2001: Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. *Plant Pathol* 50, 10-27.