# Progress in breeding for Crown Rust (*Puccinia coronata* f. sp. *lolii*) resistance in perennial ryegrasses

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## Abstract
Crown rust (*Puccinia coronata* f. sp. *lolii*) is a major disease of perennial ryegrass (*Lolium perenne*), causing reduced forage yield, plant rooting depth, sward content in mixed pastures, animal intake and digestibility and is associated with animal health problems such as facial eczema. The crown rust resistance (CRR) of contemporary perennial ryegrass cultivars commercially available in Australia has not been studied. Utilising CRR data collected in nine trials over an eight-year period at Ballarat, Victoria, the CRR of 48 commercially marketed varieties were explored. Significant ($p<0.001$) variation for CRR was identified between marketed perennial ryegrass varieties and the CRR ranking determined for older cultivars and ecotypes was consistent with available literature. Large variation was found in varieties of varying germplasm origins and improvements over older varieties was not large. We suggest progress for this trait may have been temporarily checked by the industries transition to new germplasm sources for other benefits. On average, breeding lines were superior to commercial varieties indicating future improvements are likely. The results of this study suggest that an independent CRR could be easily developed to assist producers choosing between marketed cultivars.

Key words: *Lolium perenne*, plant breeding, robust resistance

## Introduction
Perennial ryegrass has high dry matter production, comparatively high forage quality and strong grazing recovery (NSW DPI, 2019); unsurprisingly it is one of the most widely used forages in temperate Australian grazing systems. However, one disadvantage is its susceptibility to infection by crown rust (*Puccinia coronata* f. sp. lolii), a fungal disease that affects foliage by depriving the plant of its nutrients in a biotrophic relationship and damaging photosynthetic area.

### *On-farm impact of crown rust*
Crown rust has been estimated to cause annual losses of AUD $6.1 million (CPI adjusted to 2018) in pasture systems (Sloane et al. 1988). Proliferation of irrigated pastures since 1988 may make this a conservative estimate in the current context. The first quantification of agronomic losses from the disease was in New Zealand by Lancashire and Latch (1966), who demonstrated reduction in: forage yield (up to 53 %); tiller density (up to 20 %); and root weight (26 %). Plummer et al. (1990) reported crown rust to cause reduced root weight, leaf area and tiller density and increased tiller deaths, especially in younger tillers.

The crown rust pathogen creates a water-soluble carbohydrate (WSC) sink in the plant causing reduced growth and forage quality; a 10 % leaf area infection can cause a 50 % decline in WSC concentration (Carr 1975). Water-soluble carbohydrate concentrations are positively correlated with dry matter digestibility (Humphreys 1989) and efficient rumen fermentation (Grimes et al. 1967) so crown rust likely reduces both of these factors. Furthermore, the pathogen causes reduced forage palatability and grazing avoidance leading to a build-up of dead matter and consequently under-utilisation by grazing animals (Carr 1975). Increased dead matter is associated with the build-up of saprophytes (McKenzie 1971) associated with animal health issues such a facial eczema in sheep and cattle.

### *Crown rust resistance in perennial ryegrass*
Studies report variation in crown rust resistance (CRR) between perennial ryegrass cultivars and experimental/breeding lines (Clarke et al. 1997) and demonstrate that CRR is subject to temporal variation (Critchett 1991). Environment plays a large role in crown rust infection in perennial ryegrass with optimal conditions for infection believed to be a 16 to 18 hour photo-period at 18-20 °C and high humidity (Critchett 1991).

Tetraploid ryegrasses are suggested to be more resistant than diploid ryegrasses (Armstrong and Rumball 1976) and the inheritance of CRR is both quantitative and qualitative (Dracatos et al. 2008).

Genetic diversity of *Puccinia coronata* f. sp. *lolii* were compared between New Zealand and Victoria, Australia (Dracatos et al. 2008) and significant genetic diversity was identified within and between Victorian regions; contradictory to New Zealand where little variation was identified between North and South Island isolates (Dracatos et al. 2008). Arojju et al. (2018) demonstrated that infecting strains of *Puccinia coronata* f. sp. *lolii* adapt over short periods of time causing once resistant plants to become susceptible.

As best as we can tell, no Australian publications have systematically addressed varietal differences in the rust resistance of marketed perennial ryegrasses since Clarke et al. (1997) when approximately 95 % of all certified perennial ryegrass seed produced in Australia was crown rust susceptible northern European derived, Australian naturalised ecotypes such as 'Victorian' and 'Kangaroo Valley' (Cunningham et al. 1993). The marketplace has since shifted toward proprietary cultivars in which improved crown rust resistance is reported (Arojju et al. 2018). In this study, we aim to report on the rust resistance of perennial ryegrasses marketed in Australian since this time.

**Method**

Each autumn in Ballarat (-37°56'S, 143°95'E)) in south-west Victoria between 2010 and 2018, PGG Wrightson Seeds sowed a single row and plot perennial ryegrass trial containing commercial cultivars and experimental varieties as part of a broader testing network. Trials were a randomised complete block design with three or four replicates. The soil is a deep red Krasnozem weathered in-situ from basalt and was irrigated through summer and managed under a combination of mowing and sheep grazing for three years. Crown rust infection was scored whenever observed, usually through the summer and autumn months, using a scale between nine (no crown rust) and one (very high crown rust infection). A total of 13 rust observations were recorded. The data was analysed using a linear mixed model in DeltaGen (Jahufer et al. 2018) with cultivar/variety fitted a random effect and year and season as a fixed effect. This multi-year trialling allows greater understanding of CRR in different years at this location and under varying environmental conditions. In total, BLUPs were generated for 48 commercial lines and 447 experimental/breeding lines.

**Results**

While severity of rust infection did vary significantly between years ($p<0.05$), no significant year by variety or season by variety interaction could be identified. Variety CRR was found to vary significantly ($p<0.001$) and the mean broad sense heritability of CRR at this location averaged 87.2 %. The mean CRR BLUP score for commercial cultivars was 6.44 compared to 7.04 for experimental/breeding varieties.

**Discussion**

The greatest rust infection was observed to be in 2011 (data not shown) as it had an unusually wet summer with average warmth. Crown rust was only observed in late summer and autumn, the time of year most likely within the optimal conditions for crown rust infection described by Critchett (1991).

Where the same varieties occur in our study as in Clarke et al. (1997), varieties ranked similarly. For instance, ecotype Victorian is the most susceptible commercial variety to crown rust we trialled and Clarke et al. (1997) identified it as highly susceptible in all trialled locations. Similarly, our findings also agree that Mangere germplasm derived New Zealand cv. Nui (Stewart 2006) has improved rust resistance compared to ecotype Victorian.

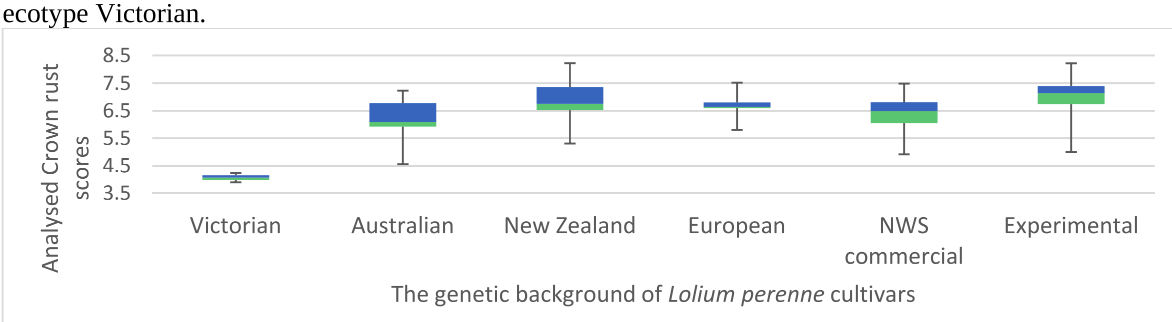

**Figure 1. The analysed CRR score based on the perennial ryegrass genetic origin compared to experimental/breeding lines of PGG Wrightson Seeds breeding programs.**

While almost all commercial cultivars are significantly improved for CRR as compared to ecotype Victorian, (where rust BLUP was 3.9), Figure 1. displays a wide range of variation exists for CRR of commercial cultivars (4.6 through to 8.2). The industry in Australasia has transitioned towards North West Spanish genetics, seeking the benefits of out of season yield (Stewart 2006, Harmer et al. 2016), however these also have a large range of CRR, as do the modern European varieties trialled. It is possible that gains in CRR up to this point have been constrained by a change in germplasm origin (towards North West Spanish) for good reason. With exploitation being relatively recent, most may only be a few generations breeding from wild accessions. Another explanation for the noted general lack of improvement may be strains of crown rust adapting (Arojju et al. 2018), although consistent ranking of varieties between 1996 (Clarke et al. 1997) and now would not support this.

The data set contained 448 experimental varieties whose CRR average was significantly greater ($p<0.0001$) than commercial cultivars and ecotypes. This suggests farmers might expect future improvements in this trait. Genotype by environment and genotype by rust-isolate interaction is known to occur for CRR (Clarke et al. 1997, Fè et al. 2016) and is not addressed by this single location analysis. A broader analysis of other testing locations throughout Australia and New Zealand is recommended to investigate the G x E interaction in CRR in perennial ryegrass in Australasia. It may transpire that the development of an Australasian CRR index or regional indexes is feasible; this information could complement the existing forage yield based independent cultivar selection tools developed to support farmers, i.e. the Forage Value Indexes (Dairy Australia and Dairy NZ) and the Pasture Trial Network (Meat and Livestock Australia).

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