Virulence patterns of oat crown rust in Australia - season 2022

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Abstract

Puccinia coronata f. sp. *avenae* (*Pca*) is an important foliar pathogen of oat which causes crown rust disease. The virulence profile of 48 *Pca* isolates derived from different locations in Australia was characterised using a collection of oat lines often utilised in rust surveys in the USA and Australia. This analysis indicates that *Pca* populations in Eastern Australia are broadly virulent, in contrast to the population in Western Australia (WA). Several oat lines/*Pc* genes are effective against all rust samples collected from WA, suggesting they may provide useful resistance in this region if deployed in combination. We identified 19 lines from the US oat differential set that display disease resistance to *Pca* in WA, some in agreement with previous rust survey reports. We adopted the 10-letter nomenclature system to define oat crown rust races in Australia and compare the frequency of those virulence traits to published data from the USA. Based on this nomenclature, 42 unique races were detected among the 48 isolates, reflecting the high diversity of virulence phenotypes for *Pca* in Australia. Nevertheless, the *Pca* population in the USA is substantially more broadly virulent than that of Australia. Close examination of resistance profiles for the oat differential set lines after infection with *Pca* supports hypotheses of allelism or redundancy among *Pc* genes or the presence of several *R* genes in some oat differential lines. These findings illustrate the need to deconvolute the oat differential set using molecular tools.

Manuscript body

Oat crown rust disease is caused by the basidiomycete fungus *Puccinia coronata* f. sp. *avenae* (*Pca*) (Nazareno et al. 2018). This disease is prevalent in oat growing regions across Australia and worldwide, raising the profile of this pathogen as one of global importance. Oat is important to Australia's economy as it is used for milling, grazing, and feed hay (Cowman et al. 2021). Australia has a leading position in the production of high-quality milling oats, and in the 2021-2022 cropping season produced 1.7 million tonnes of oat (Australian Bureau of Statistics 2021). Regions known for their production of milling oat include Western Australia (WA), the Eyre and York Peninsulas of South Australia, Western and North-eastern Victoria, and the Riverina and Central New South Wales (NSW). Forage oats are widely grown in Central and South Queensland and Northern NSW and to some extent in WA. Oat production in WA is heavily focused on grain for feed and hay. It is estimated that 48% of Australia's hay export comes from WA alone (Department of Primary Industries and Regional Development 2017). Given the negative impact of crown rust on plant growth, yield, grain weight, and palatability, the natural populations of *Pca* must be closely monitored to detect changes in virulence traits. The recent increases in market demand for Australian oats have justified additional research and development investments to support the industry (Cowman et al. 2021).

The evolution of virulence in *Pca* is driven by an arms race between the pathogen and the host (Nazareno et al. 2018). The boom-and-bust cycle illustrates this process, as the pressure exerted by the extensive adoption of an oat cultivar with a disease resistance (*R*) gene leads to the selection of rare variants in the pathogen that can overcome that specific resistant trait. This results in a frequency increase of that virulence trait in the rust population (Figueroa et al. 2023). Consequently, planting

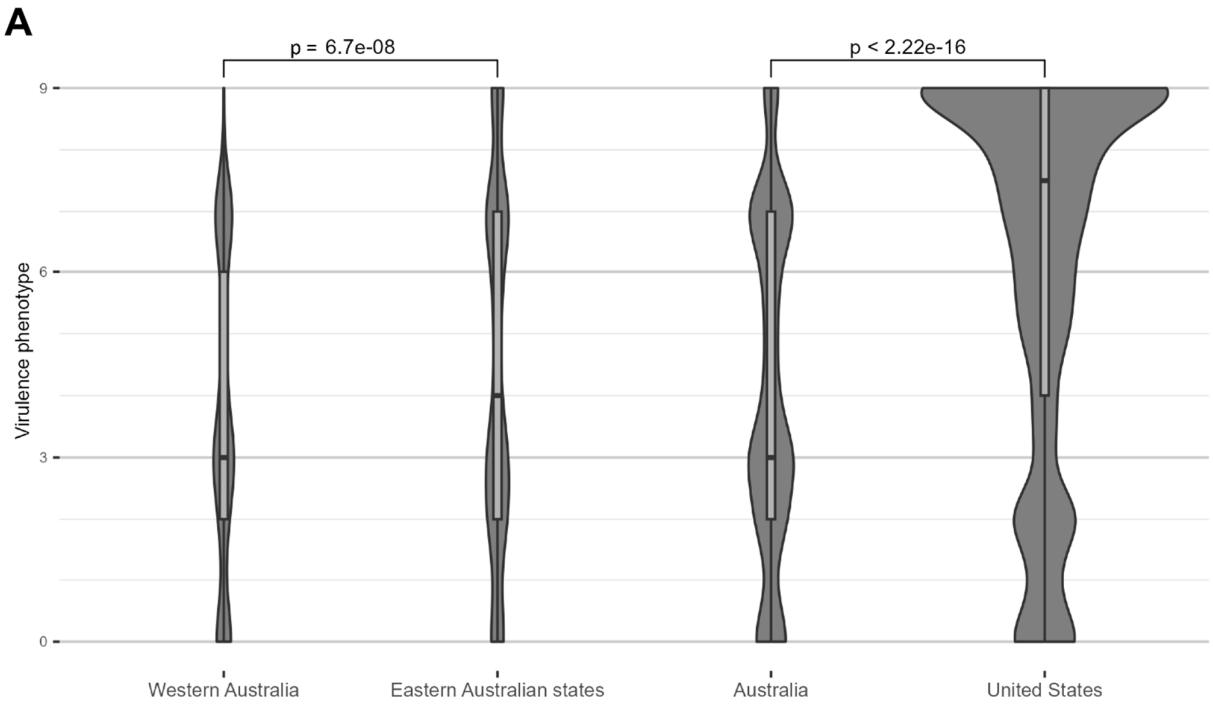
of that oat cultivar may become less common, as it is no longer resistant to the pathogen. Oat resistance to *Pca* follows the gene-for-gene concept initially described by Flor (1971), which is now more broadly understood to involve recognition of the pathogen by the plant immune system (Dodds 2023; Pitsili et al. 2020). Plant *R* genes generally encode intracellular immune receptor proteins mostly belonging to the nucleotide binding leucine rich repeat receptor (NLR) class. These receptors recognise pathogen 'effector' proteins, known as virulence (Avr) factors, that are delivered into host cells during infection to suppress host basal defences and facilitate infection (Figueroa et al. 2021; Petre et al. 2014). This type of resistance is known as race-specific resistance, which provides the framework utilised by plant pathologists to nominate races (pathotypes) of a pathogen. These pathogen races represent specific virulence profiles on a set of selected cultivars that collectively are referred to as a host differential set. At the molecular level, it is the combination of Avr factors (effectors) that defines a race. Each of the differential cultivars preferably contains a single race-specific *R* gene; however, that is often difficult to achieve due to the time commitment and investment required to generate isogenic lines and develop molecular markers to ensure *R* gene presence. Oat differential sets are used extensively to categorise *Pca* races (pathotypes) but differ substantially in their composition in different regions (Carson 2011; Chong et al. 2000; Nazareno et al., 2018).

Through a network of plant pathologists and industry representatives, we received and processed samples for 48 *Pca* isolates derived from oat producing areas in Australia (WA:20, NSW:11, VIC:9, SA:4, QLD:4) to investigate the virulence landscape of this pathogen in the country and create a foundational resource for future studies (**Table 1**). Rust samples were received as infected foliar tissue material and

recovered by inoculation onto the widely susceptible cultivars Swan or Marvelous. Single pustule isolates were collected and amplified using standard techniques to determine virulence profiles (Miller et al. 2020). Rust isolate stocks are kept at –80°C for long term storage. Presently, there is no universal oat differential set or nomenclature system to facilitate an international monitoring system. Thus, we adopted an oat differential set utilised for annual rust surveys in the USA and added several oat cultivars that are often included in rust surveys conducted by Australian Cereal Rust Control Programme (**Table S1**). Oat lines from the USA were imported from the USDA-ARS (St. Paul, MN, USA) to the CSIRO's quarantine facility (Black Mountain Laboratories, Canberra, Australia). The remaining oat lines were accessed through the Australian Grains Genebank (AGG) or breeders. A single seed descent increase was undertaken for each oat line using standard growth conditions; briefly, plants were grown at 23°C for 16 hours light and 18°C for 8 hours dark and fertilized at stem elongation and anthesis with Osmocote® 19-9-10+2MgO+TE all-purpose slow release (Everris International B.V., Heerlen, Netherlands). To determine virulence profiles, *Pca* isolates were inoculated onto the differential lines and infection scores were recorded at 12 dpi with race assignments (pathotypes) made using the North American 10-letter nomenclature (Carson 2011; Chong et al. 2000; Nazareno et al. 2018). Infection scores were converted to a 0-9 numeric scale for statistical comparisons (https://data.csiro.au/collection/csiro:58613) (Miller et al., 2018, 2020).

Overall, the *Pca* population from Eastern Australia (NSW, QLD, VIC, and SA) is more broadly virulent compared to the population in Western Australia (**Figure 1A**; Wilcoxon test $p = 6.7 \times 10^{-8}$). Collectively, *Pca* isolates from WA are only virulent on 21 of 40 USA differential lines, while isolates from Eastern Australia have virulence to 38 of 40 US differential lines (**Figure 1B**). We identified 19 lines from the US differential

set that display resistance to all the *Pca* isolates collected from WA. Virulence to 18 lines, including Pc91, Pc94, and Pc96, was only sampled in Eastern Australia. Across all lines tested, the most broadly virulent isolates were from eastern Australia (22QLD116, 22NSW123, 22NSW79), while the least broadly virulent isolates were from western and southern Australia (22WA22, 22VIC01, 22SA10, 22VIC49) (**Figure 1B**). Rust isolates from QLD had the highest average virulence on all lines tested (5.47), followed by those from NSW (4.59), VIC (4.35), SA (4.28), with those from WA being least virulent on average (3.75). We obtained 42 unique 10-letter races for the entire *Pca* collection (48 isolates), illustrating the phenotypic diversity of the pathogen in Australia (**Table 1**).


A
p = 6.7e-08
p < 2.22e-16
Virulence phenotype
9
6
3
0
Western Australia
Eastern Australian states
Australia
United States

B
22WA02–01
22WA09–01
22WA15–01
22WA22–01
22WA25–01
22WA27–01
22WA29–01
22WA30–01
22WA31–01
22WA43–01
22WA45–01
22WA46–01
22WA47–01
22WA50–01
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22SA12–01
22SA93–01
22VIC01–01
22VIC117–01
22VIC126–01
22VIC128–01
22VIC129–01
22VIC49–01
22VIC67–01
22VIC71–01
22VIC74–01
22NSW103–01
22NSW107–01
22NSW121–01
22NSW123–01
22NSW38–01
22NSW48–01
22NSW75–01
22NSW76–01
22NSW79–01
22NSW84–01
22NSW97–01
22QLD110–01
22QLD114–01
22QLD116–01
22QLD118–01
Pc14
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Pc94
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Marvelous
H548
IA.B605Xsel.
WIX4361.9
TAM.O.405
Belle
Hifi
Leggett
Stainless
Swan
Ukraine
Santa.Fe
Trispernia
Saia
Cleanleaf
Barcoo
Volta
Gwydir
Warrego
Culgoa
Bondvic
X716
X534
Bettong
Genie
Landhafer
Virulence
0 to ;C
1; to 2
3 to 4

Figure 1. Virulence profile of a subset of 48 Australian *Puccinia coronata* f. sp. *avenae* (*Pca*) isolates from across the country. **A)** Violin plot illustrating cumulative difference in virulence on the 40 US differential lines for 48 Australian and 152 US *Pca* isolates, and between Australian isolates from Western Australia (WA) and Eastern Australia (QLD, NSW, SA, and VIC). The *p*-value is the result of a two-sided Wilcoxon rank sum test of numeric phenotype scores on the US differential set. Violin plots were generated in R using package ‘ggplot2’ and the two-sided Wilcoxon rank sum test was performed and integrated into the plot using R package 'ggpubr’ (Wickham 2016; Kassambara 2023). **B)** Heatmap showing virulence profiles of isolates collected in 2022 (x-axis) on US differential lines and cultivars used in Australian surveys (y-axis). High infection scores are shown in red indicating high virulence (susceptibility) and lower scores (yellow/orange) indicate avirulence (resistance). Heatmap was constructed using the R package ‘ComplexHeatmap’ (Gu 2016).

Virulence scores on 19 of the 40 US differential lines can be compared with survey information from the Australian Cereal Rust Program (ACRCP) as they have been included in previous surveys (Australian Cereal Rust Survey 2020, 2021, 2022; Cuddy et al. 2016; Cuddy and Park 2014; Park 2013; Park and Kavanagh 2002, 2003, 2008, 2009, 2011; Park and Whale 1999). Of these, disease resistance scoring on 13 lines are consistent with reports from 1998-2022 by the ACRCP (Pc36, Pc39, Pc46, Pc50, Pc51, Pc56, Pc59, Pc61, Pc64, Pc68, Pc91, H548, WIX4361-9). Historically, virulence was detected in all 19 lines in Eastern Australia, but our study did not sample virulence for Pc58 or Pc63 in this region (Cuddy et al. 2016; Cuddy and Park 2014; Park and Kavanagh 2009). We identified 12 lines which were resistant to all of the WA isolates in our sample, eight of which agree with historic survey records (Pc36, Pc50,

Pc56, Pc59, Pc63, Pc68, Pc91, WIX4361-9), since virulence to these lines has never been recorded in WA. Virulence to the other four oat lines (Pc38, Pc52, Pc55, Pc71) has only been detected rarely in WA (Australian Cereal Rust Survey 2021, 2022; Park 2000), which may explain why no virulence was detected in this limited sample.

We also tested 17 oat lines often included as part of the ACRCP oat crown rust surveys (Brake et al. 2001; Park 2013). Six of these (Swan, Ukraine, Santa Fe, Trispernia, Bondvic, Landhafer) were susceptible to most *Pca* isolates from all Australian states. The *Pc* genes postulated to exist in these lines include *Pc3c*+*Pc4c*, *Pc4*, *Pc5*, *Pc6*, *Pc6c*, *Pc6d*, *Pc7*, *Pc8*, *Pc9*, and *Pc21*, which were deployed in the field more than 50 years ago (Nazareno et al. 2018; Simons 1985). As such, these lines are not viable sources of crown rust resistance and could be excluded from future crown rust surveys as they are not informative. Importantly, among the Australian lines, the cultivar Barcoo was the only genotype that was effective against all examined *Pca* isolates. However, according to historic records *Pca* overcame Barcoo's resistance in 2001 (Park 2013), although this virulence trait seems to exist at a low frequency (only 1 or 2 samples each year) in NSW and QLD populations (Park 2013; Park and Wellings 2010; Park et al. 2022). There were eight other cultivars that displayed resistance to all WA isolates (Culgoa, Bettong, Cleanleaf, Saia, Volta, Warrego, Genie, Gwydir), consistent with survey data which detected no virulence to these lines in WA isolates (Australian Cereal Rust Survey 2020, 2021, 2022). The oat cultivar Cleanleaf is postulated to carry genes *Pc38*, *Pc39*, and *Pc52* (Park 2013), so our findings are consistent as we did not detect virulence on the Pc38 or Pc52 lines when testing Pca isolates from WA. However, the first report of crown rust virulence to Cleanleaf in eastern Australia (NSW and QLD) dates back to 1995 (Park et al. 2000). Similarly rust survey records indicate that virulence for cultivar Warrego was

first identified in 1998, and in 2001 virulence on Gwydir and Bettong also emerged (Park 2013). Virulence on cultivars Genie and Volta are reported for years 2008 and 2010, respectively. These virulence traits are present in the pathogen populations of eastern Australia; therefore, it is possible for their frequency to increase or for virulent isolates to migrate to the west of the country.

Finally, we compared the phenotypic data for the Australian *Pca* isolates to data for 152 US isolates collected between 2015-2018 (Hewitt et al. 2023; Miller et al. 2020) to compare with Australian data. As illustrated by the violin plots (**Figure 1A**), the 2022 Australian *Pca* collection is significantly less virulent than the US collection from 2015-2018 (mean virulence 3 and 7.5, respectively; Wilcoxon test $p < 2.2 \times 10^{-16}$). By comparing virulence profiles between Australian *Pca* in this study and *Pca* from the US (Hewitt et al. 2023; Miller et al. 2020), we noted that certain differential lines have already lost resistance in the US but were still mostly effective in Australia (WIX4361-9, Belle, Stainless). Furthermore, the oat differential lines named Pc94 and Leggett, which is postulated to carry Pc68 and Pc94, still exhibit broad resistance in both countries at present, although we expect virulence evolution is already occurring in both the USA and Australia due to evolutionary pressure exerted by the use of these *R* genes.

Two GWAS analyses of virulence in *Pca* have indicated complex relationships among *Pc* genes represented in the oat differential sets (Hewitt et al. 2023; Miller et al. 2020). For instance, the positive association previously reported for *Pc48* and *Pc52* virulence by Chong and Zegeye (2004) in combination with GWAS results by Hewitt et al. (2023) for isolates in the USA and South Africa suggest that *Pc48* and *Pc52* recognise the same *Avr* locus and may be alleles of the same gene. Interestingly, the oat lines Pc48 and Pc52 show similar patterns of resistance but do not entirely match;

this suggests that they have alleles with different specificities, or one or both lines have multiple *R* genes. Noticeably, we observed highly similar resistance profiles for Pc39, Pc38, Pc55, Pc70, and Pc71 in our infection assays. Previously, the *R* genes *Pc*39, *Pc*55, and *Pc*71 have been postulated as being either allelic or the same gene by various authors (Chong and Seaman 1989; Kiehn et al. 1976; Leonard et al. 2005). Moreover, GWAS results (Hewitt et al. 2023; Miller et al. 2020) for USA *Pca* isolates detected virulence associations for Pc38, Pc39, Pc55, Pc63, Pc70, and Pc71 in the same genomic interval, suggesting that some of the immunoreceptors encoded by these genes recognise the same *Avr* effector or genetically linked *Avr* effectors. Thus, data derived from the Australian *Pca* infections is consistent with this scenario. Furthermore, our results support the hypothesis by Hewitt et al. (2023) that the oat differential lines Pc62 and Pc64 carry alleles of the same *R* gene as the resistance profiles of these lines are highly similar. The resistance phenotypes of the lines Pc35 and Pc58 also agree with the results from Hewitt et al. (2023), suggesting that the oat lines Pc58 and Pc35 carry one *R* gene in common and the Pc58 line contains at least one additional *R* gene. As highlighted previously by Hewitt et al. (2023), we also observed consistency between the resistance profiles of the oat differential line Pc91 and the oat cultivar Hi-Fi, which was derived from Amagalon, the original source of *Pc91* (McMullen et al. 2005). In summary, our findings provide additional motivation for the need to develop an oat differential set that clearly differentiates among *R* genes and eliminates redundancy.

The expansion of the oat crown rust collection in the coming years coupled with population genomics analysis will provide an accurate representation of the pathogen's genetic diversity. Recent advances in generating genome references of *Pca* (Miller at al. 2018) including a nuclear-phased chromosome-level assembly

(Henningsen et al. 2022) provide a strong foundation to study the genetic relationships of these isolates. Such information will be instrumental in determining the factors contributing to host adaptation of *Pca* in Australia. Rust fungi are known to evolve virulence by mutation, reassortment of virulence alleles, and somatic hybridisation (Figueroa et al. 2020). The invasive species *Rhamnus cathartica*, also known as common buckthorn, acts as an alternate host for *Pca* and allows sexual reproduction (Nazareno et al. 2018). Several studies (Berlin et al. 2018; Hewitt et al. 2023; Miller et al. 2020; Zhao et al. 2016) document the influence of sexuality in the diversity of the oat crown rust pathogen. In Australia, a sexual host for *Pca* has not been reported (Burdon and Thrall 2008), so sexual reproduction is not expected to play a role in the evolution of *Pca* in Australia, suggesting the Australian population is evolving by clonality and stepwise mutation. Wild oats are abundant in the Australian landscape and serve as an additional host for *Pca*, which is predicted to favour mutations and contribute to the emergence of diversity (Burdon and Thrall 2008). However, it is not possible to define lineages from the phenotypic data alone; more detailed genotypic analysis will be required to fully characterize the number and diversity of clonal lineages present in the Australian *Pca* population.

The findings from this study, along with recent research (Hewitt et al. 2023; Miller et al. 2020) highlight the importance in developing durable crown rust resistance in oat. This can be achieved by stacking the most current effective genes, including both APR (Adult Plant Resistance) and ASR (All Stages Resistance) (Periyannan et al. 2017) and identifying and integrating novel sources of resistance into the oat breeding pools (Figueroa et al. 2020; Klos et al. 2017; Nazareno et al. 2018, 2023).

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Table 1. List of *Pca* isolates collected across Australia.

Isolate[1]	City	State	Collection date	10-letter race (pathotype)
22VIC01-01	Melbourne	VIC	01/09/2022	BFDQLHBCQB
22WA02-01	Geraldton	WA	09/09/2022	BDLQLBLCQB
22WA09-01	Greenough	WA	13/09/2022	BFLGMBBCQB
22SA10-01	Virginia	SA	20/09/2022	BDQGCBBCQB
22SA11-01	Barabba	SA	20/09/2022	BKQQCLBCQB
22SA12-01	Mallala	SA	20/09/2022	BFDQLMBCQB
22WA15-01*	Corrigin	WA	01/10/2022	BFBQLBBCQB
22WA22-01	Karranadgin	WA	06/10/2022	BKDGLHBCQB
22WA25-01*	Watercarrin	WA	06/10/2022	BFBQLBBCQB
22WA27-01	Bruce Rock	WA	06/10/2022	BFDQLCLCQB
22WA29-01	Hyden	WA	06/10/2022	BDLQCCBCQB
22WA30-01	Holt Rock	WA	12/10/2022	BFLQMBBCQB
22WA31-01**	Myalup	WA	12/10/2022	BFLGLBBCQB
22NSW38-01	Wagga Wagga	NSW	14/10/2022	MQBSBBPFQB
22WA43-01	Perth City	WA	14/10/2022	LFDGLRBCQB
22WA45-01	Dumbarton	WA	13/10/2022	BKLQCCLCQB
22WA46-01	Dale	WA	13/10/2022	BJLQBCLCQB
22WA47-01	Northam	WA	13/10/2022	BFBQLMBCQB
22NSW48-01	Wagga Wagga	NSW	21/10/2022	MQBNBBPFLB
22VIC49-01****	Horsham	VIC	17/10/2022	BFDGLHBCQB
22WA50-01	Cascade	WA	06/10/2022	QJBQRBLCQB
22WA53-01	Highbury	WA	19/10/2022	BDBQBCBCQB
22WA54-01	Roelands	WA	19/10/2022	GDLQBCBCQB
22WA55-01	Dalwallinu	WA	21/10/2022	BFBBBBBCLB
22VIC67-01****	Trawalla	VIC	26/10/2022	BFDGLHBCQB
22VIC71-01	Culgoa	VIC	28/10/2022	BFBGLMLCGB
22VIC74-01****	Dookie	VIC	28/10/2022	BFDGLHBCQB
22NSW75-01	Brocklesby	NSW	28/10/2022	BFDQBHBCGB
22NSW76-01****	Holbrook	NSW	28/10/2022	BFDGLHBCQB
22NSW79-01	Balranald	NSW	30/10/2022	TQRPLLTFLB
22NSW84-01	Young	NSW	02/11/2022	BFDGBMBCLB
22WA86-01	Wialki	WA	30/09/2022	BDLGMBBCQB
22WA88-01**	Gibson	WA	09/11/2022	BFLGLBBCQB
22WA89-01	Gibson	WA	09/11/2022	BPLQMBNCQB
22SA93-01	Urrbrae	SA	21/10/2022	MSBJLLSMQG
22NSW97-01***	Canowindra	NSW	03/11/2022	GBBQBBLFLB
22NSW103-01	Coolah	NSW	03/11/2022	TQMPNLTPMR
22NSW107-01	Bellata	NSW	04/11/2022	BBBQBBBFLB
22QLD110-01***	Goondiwindi	QLD	04/11/2022	GBBQBBLFLB
22QLD114-01	Kingaroy	QLD	14/11/2022	FLMPLLKTLJ
22QLD116-01	Toowoomba	QLD	14/11/2022	PQPPLLTPLG
22VIC117-01	Jeparit	VIC	08/11/2022	HLPNDLTFNB
22QLD118-01	Warwick	QLD	16/11/2022	TQRPMLTKLD
22NSW121-01	Wagga Wagga	NSW	-	BCDQLHBCQB
22NSW123-01	Heatherbrae	NSW	07/10/2022	TQMPLLTPMR
22VIC126-01	Horsham	VIC	11/11/2022	RQMSLLTFLB
22VIC128-01	Crowlands	VIC	07/11/2022	GBBRBBLFLB
22VIC129-01	Elmhurst	VIC	07/11/2022	CKFQMHBFQB

[1] *,**,***,**** indicate isolates with identical race codes.