Virulence and genomic analysis of *Puccinia coronata* f. sp. *avenae* in Australia identifies new races and a new lineage in 2024

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Abstract

Crown rust, caused by *Puccinia coronata* f. sp. *avenae* (*Pca*), remains a persistent threat to oat production in Australia. To monitor recent shifts in virulence and population structure, 30 *Pca* isolates collected during the 2024 growing season across major Australian oat-producing regions were analysed. Virulence analysis of 30 isolates using 52 oat differential lines identified 25 unique races that were not detected in previous years. Whole-genome sequencing of 28 of these isolates were analysed in the context of a broader historical Australian and international genomic datasets including isolates from Taiwan, South Africa, USA. Results confirmed the uniqueness of the Australian *Pca* population and revealed well-established genotypic lineages persisting over multiple years, with L18 and L16 being dominant. Notably, L16 was again present in Western Australia after being undetected in 2023, while L18 maintained its prevalence for a third consecutive season. Beyond these dominant groups, phylogenetic analysis and a *k*-mer containment analysis also identified a novel and genetically distinct lineage, designated as L19, represented by one isolate collected in WA. To add to the characterisation of lineage L19, we recorded virulence phenotypes on a small collection of current commercial cultivars. These findings enhance understanding of *Pca* diversity and emphasise the importance of surveillance approaches that integrate phenotypic and genomic surveillance.

Keywords: crown rust, virulence, collection, lineage, oat

37 **Manuscript body**

38 Oat crown rust (OCR), caused by *Puccinia coronata* f. sp. *avenae* (*Pca*), is the most destructive
39 foliar disease for oat production worldwide (Nazareno et al. 2018). The disease typically
40 causes yield losses of 10% - 40% under epidemic conditions (Nazareno et al. 2018). In a severe
41 outbreak, yield losses have reached as high as 50% (USDA-ARS CDL 2014). In addition to
42 reducing yield, the disease also affects grain quality and fodder value (Simons 1985), posing
43 persistent challenges to both milling and forage oat production in Australia and other
44 countries. While breeding for host resistance has proven to be effective (McDonald and Linde
45 2002), the durability of resistance genes is impacted by evolution of virulence in the *Pca*
46 population (Carson 2011; McCallum et al. 2007; Moreau et al. 2024). Therefore, ongoing
47 surveillance to detect changes in population structure and virulence profiles remains an
48 essential component of OCR management strategies (Nazareno et al. 2018).

49 In Australia, routine surveys are conducted to monitor *Pca* virulence patterns and evaluate the
50 effectiveness of *Pc* resistance genes (Henningsen et al. 2024b; Nguyen et al. 2025; Park et al.
51 2025). The high phenotypic diversity among *Pca* isolates is well-supported, with dozens of
52 unique races identified each year (Henningsen et al. 2024b; Nguyen et al. 2025; Park et al.
53 2025). These findings underscore the dynamic nature of the *Pca* population. So far, a total of
54 18 genotypic lineages of *Pca* based on genome-wide variants have been characterised using
55 137 samples collected between 2020 and 2023 (Henningsen et al. 2024a). This framework
56 enables a consistent lineage assignment and together with extensive haplotype resolved
57 pangenome resources (Henningsen et al. 2024a) supports epidemiological inferences about
58 migration and genetic recombination of *Pca* in Australia. Furthermore, a recent study
59 highlights the need to complement phenotype-based assessments with genomic data as *Pca*
60 isolates with similar virulence profiles can belong to genetically distinct lineages (Henningsen
61 et al. 2024a). Therefore, full characterisation of the phenotypic and genotypic variability of
62 *Pca* is critical to optimise genetic resistance deployment and understanding phenotypic shifts
63 in the pathogen population that can impact the oat industry.

64 In this study, we report the virulence phenotypes of a collection of *Pca* isolates derived from
65 2024 and combine it with genotypic assignments based on whole genome sequencing. To
66 bring these data into a global context, we leveraged published genomic data of historical and

67 more recent international *Pca* populations (Henningsen et al. 2024a; Hewitt et al. 2024; Ho et
68 al. 2025; Miller et al. 2020). Through a national network of plant pathologists and industry
69 representatives, we obtained a total of 38 samples of *Pca* collected in 2024. These samples
70 originated from New South Wales (*n*=7), Victoria (*n*=2), South Australia (*n*=5) and Western
71 Australia (*n*=24) and were collected from wild oats (*n*=36), an unknown cultivated line (*n*=1),
72 and an oat cultivar present in a field trial at Wagga Wagga, NSW. We note the lack of *Pca*
73 sampling from Queensland, and low representation from New South Wales and Victoria, likely
74 due to dry weather conditions (Bureau of Meteorology 2025,
75 https://www.bom.gov.au/climate/maps/rainfall). Of the 38 *Pca* samples, 30 (NSW=6, VIC=2,
76 SA=5 and WA=17) were revived after inoculation on the highly susceptible cultivars Swan and
77 Marvelous following standard protocols (Miller et al. 2020). We proceeded with single pustule
78 purification keeping purified *Pca* isolates for long term storage at -80°C (Henningsen et al.
79 2024a; Miller et al. 2020).

80 Virulence phenotypes of the 30 revived *Pca* isolates from the 2024 collection were determined
81 using a panel of 52 differential lines as previously described (Henningsen et al. 2024b; Nguyen
82 et al. 2025) (**Supplemental Table 1; Figure 1A**). A total of 25 unique races according to the 10-
83 letter race coding system (Carson 2011; Chong et al. 2000; Nazareno et al. 2018) were
84 identified (**Table 1**), confirming previous observations of the high diversity of virulence
85 phenotypes for *Pca* in Australia. Two races were represented by more than one isolate:
86 "BFBGMBDCG-" consisting of five isolates: 24WA12, 24WA13, 24WA14, 24WA22 and 24WA25,
87 and "BJLQCCDCG-" consisting of two isolates: 24WA10 and 24WA23. All 25 races in 2024
88 represent new records according to previous collections (Henningsen et al. 2024b; Nguyen et
89 al., 2025). We compared the distribution of virulence scores for the 2024 *Pca* isolates with
90 those from previous years (Henningsen et al. 2024b; Nguyen et al. 2025) (**Supplemental**
91 **Figure 1**). Overall, the collection from 2024 shows a shift toward less virulence than in the
92 previous years 2022 and 2023 (p-value <0.001, Wilcoxon rank sum test) with 26 out of 52 oat
93 differential lines showing less infection scores in 2024. The oat differential lines that were
94 susceptible to the most isolates in this collection include Pc45 (*n*=29), Pc54 (*n*=29), Pc60
95 (*n*=26), Pc70 (*n*=25), and Pc46 (*n*=23). The oat lines Barcoo and Pc63 remained resistant to all
96 isolates across these seasons, and observations from 2024 support low virulence for several

97 oat lines such as Pc58, Pc59, Pc63 and WI X4361-9 mentioned in previous years (Henningsen
98 et al. 2024b; Nguyen et al. 2025).

99 To further explore population structure of the 2024 *Pca* isolates, whole-genome sequencing
100 (WGS) data was generated. Genomic DNA was extracted for each isolate from 20–40 mg
101 urediniospores using the OmniPrep Genomic DNA Isolation Kit (G-Biosciences) and
102 sequencing libraries were prepared using the Illumina DNA PCR-Free Library Preparation
103 Protocol. Sequencing on an Illumina NovaSeq X platform (150 bp paired-end reads, 15–30×
104 coverage) was completed at the Biomolecular Resource Facility, Australian National University
105 (Canberra, Australia). Two isolate samples (24SA30 and 24NSW39) did not pass WGS quality
106 filters, and the remaining 28 samples were retained for the subsequent analysis (NSW=5,
107 VIC=2, SA=4, WA=17) (**Supplemental Table 2)**. A Maximum Likelihood (ML) phylogenetic
108 analysis was conducted as described previously (Henningsen et al. 2024a) on a total of 380
109 isolates including the 28 newly sequenced isolates from 2024 and a reference panel of 352
110 previously sequenced isolates collected in Australia (2020 to 2023 collections), the United
111 States of America (USA), South Africa, and Taiwan (Henningsen et al. 2024a; Hewitt et al. 2024;
112 Ho et al. 2025; Miller et al. 2020) (**Figure 2**). The inclusion of *Pca* datasets from other
113 continents allowed a comparative genomic framework to place the 2024 *Pca* isolates within
114 both national and global contexts (Henningsen et al., 2024a). As noted by Henningsen et al.
115 (2024a) the Australian *Pca* collection presents a drastic differentiation from other countries
116 and this pattern was also observed in the samples collected in 2024. Most *Pca* isolates from
117 2024 clustered within the previously established genotypic lineages (L1 to L18). A novel
118 lineage, designated as L19, was identified from a single isolate (24WA15) collected from WA
119 (**Table 1; Figure 1A,B; Figure 3**). The isolate 24WA15 formed a distinct branch outside of the
120 18 previously defined lineages (L1–L18), with high bootstrap support (≥ 80%) and a relatively
121 long branch length. The topology of the ML phylogenetic tree indicates that L19 falls within
122 the broader group of Australian lineages (L4 to L17) that from a locally recombining population
123 derived from the L3 and L4 founder lineages (Henningsen et al. 2024a) and is most similar to
124 L7, L8, and L10, all which are present in WA. Thus, we anticipate L19 represents an additional
125 recombinant lineage and unlikely to be an exotic introduction into the continent.

126 We also assessed the distribution and frequency of genotypic lineages across Australia in the
127 2024 season and compared to the complete *Pca* collection (2022-2024) (**Figure 3A,B**). As
128 illustrated, L18 was found in all surveyed regions and accounted for 29% (8 of 28 total) of
129 samples (**Figure 1A,B; Figure 3**), consistent with our previous report of its dominant presence
130 in both 2022 and 2023 (Henningsen et al. 2024a). Although L1 has previously been reported
131 across all regions, in 2024 L1 was only sampled in NSW. We also note the representation of
132 L18 and L11 in NSW and only L18 in VIC. We only detected three genotypic lineages in South
133 Australia (L9, L16 and L18). WA harbors most of the genetic diversity, with eight lineages being
134 sampled in 2024. Although L16 was not reported 2023 after being detected in 2022, the
135 collection of 2024 showed its prominent presence, particularly in WA, where it accounted for
136 approximately one-third (9 of 28 total, and 8 of 17 from WA) of the sequenced isolates. The
137 detection of L19 adds further evidence that the *Pca* population in WA harbors extensive
138 genetic diversity that we have not yet fully captured.

139 To complement SNP-based phylogenetic inference, we additionally employed *k*-mer
140 containment analysis as an independent approach to assess genome-wide sequence identity
141 (Henningsen et al. 2024a; Sperschneider et al. 2023) to the Australian and USA pangenome
142 collection of haplotype references (Henningsen et al. 2024a). Briefly, thresholds for haplotype
143 containment were defined as ≥ 99.98 % *k*-mer identity and ≥ 99.65 % shared *k*-mers
144 (**Supplemental Table 3**). This analysis confirmed the presence of the appropriate haplotypes
145 in the 24 isolates assigned to lineages L1, L9, L11, L14, L16 and L18, and showed that L19 does
146 not contain any of the previously defined nuclear haplotypes present in the current
147 pangenome (Henningsen et al. 2024a). Thus, future expansion of the *Pca* pangenome should
148 therefore include 24WA15 to closely examine its precise origin through haplotype assembly,
149 recombination block analysis and ancestry signals.

150 Pathogenicity assays showed that 24WA15 (L19) is virulent on oat lines Pc40, Pc45, Pc46, Pc54,
151 Pc60, Pc62, Pc64, Pc67, Pc70 and IA B605Xsel in addition to the susceptible control oat lines
152 Marvelous and Swan (**Figure 1A; Table 1**). Like the other genotypic lineages present in WA
153 (e.g., L14), the virulence profile of 24WA15 is limited to a relatively small number of *Pc* genes
154 and contrasts with the broadly virulent lineage L1 common in the Eastern side of the country
155 but detected rarely in WA (Henningsen et al. 2024a) (Figure 1A; Figure 3A). To further explore

156 the industry risk of L19 we assessed the virulence profile of 24WA15 and compared it to
157 samples from L1, L2, L3, L17, and L18 across a small set of commercial oat cultivars including
158 the cultivar Swan as a susceptible control (Supplemental Figure 2). Notably, all commercial
159 cultivars were susceptible to L1, and most varieties were resistant to L2 and L17. Lineages L18
160 and L19 were similar in virulence.

161 Comparison of the genotypic and phenotypic data (Figure 1A) also highlights the role of
162 mutation in generating virulence diversity within genetic groups. Isolates within the same
163 genetic lineage differ in virulence on some differential lines likely due to stepwise mutation
164 events in specific avirulence gene loci within these clonal lineages.

165 While most *Pca* samples included in this study were collected from wild oats, we and other
166 have previously shown that there is no distinction between *Pca* populations found on wild or
167 cultivated oats (Henningsen et al. 2024a; Hewitt et al. 2024; Park et al. 2025). Wild oats are
168 extremely prevalent in Australia and are found near the edges of oat plantations, so this
169 collection represents the disease challenges present in cultivated oat fields. Whilst the 2024
170 *Pca* collection reveals some changes in pathogen diversity both at the phenotypic and
171 genotypic level, we note the sampling limitations and biases that should be considered before
172 concluding this is due to shifts in host-related selection pressures. The reported genetic
173 recombination of *Pca* in WA (Henningsen et al. 2024a) indicates involvement of a small
174 number of less virulent parental genotypes (i.e., L3 and L18) which has not led to a substantial
175 impact on the emergence of new virulent races. Nevertheless, the potential for recombination
176 to expand to additional lineages, especially the highly virulent L1 lineage, poses a risk for
177 future emergence of novel virulence combinations. Hence it is important to keep monitoring
178 for new lineages in the Australian *Pca* population. Future research will determine whether L19
179 is a short-lived variant or has the capacity to persist and spread across regions. However, the
180 detection of L19 as part of this relatively small collection warrants increased focus in
181 surveillance, particularly in WA.

182 Together, our findings highlight the value of using the *Pca* haplotype pangenome as a resource
183 to enhance pathogen monitoring such as in the case of the 2024 *Pca* survey. This study
184 demonstrates the power of coupling phenotypic and genotypic data in advancing our
185 understanding of the evolution of *Pca* population in Australia. Future surveillance efforts,

particularly in WA, will be key to assess the completeness of the current assignment of genotypic lineages and support breeding programs.

Data availability

Genomic short reads are available on the CSIRO Data Access Portal at https://data.csiro.au/collection/csiro:69616. Data underlying summary statistics and scripts used to perform analyses and generate figures are available on GitHub https://github.com/Renee-SU/Pathotyping_2024.

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Figure Legends

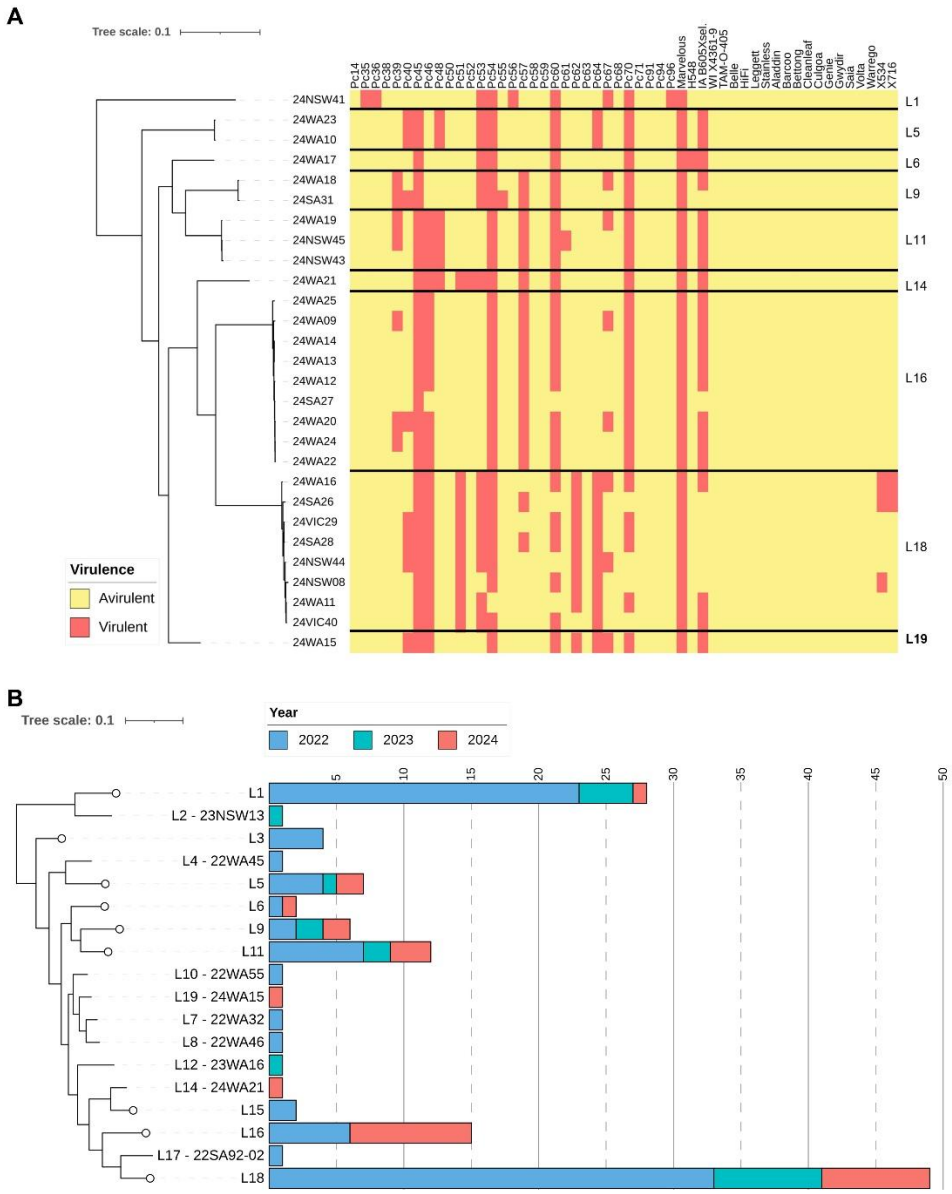


Fig. 1. Australian *Pca* isolates collected in 2024. **A**. Heatmap showing virulence profiles of *Pca* isolates collected in 2024 (x-axis) on the oat differential lines (y-axis). High infection scores indicating high virulence (susceptibility) are shown in red, and lower infection scores indicating avirulence (resistance) are shown in yellow. WA = Western Australia, SA = South Australia, VIC = Victoria and NSW = New South Wales. **B.** Bar plots showing number of *Pca* isolates collected from 2022 to 2023 (Henningsen et al., 2024b; Nguyen et al., 2025) and this study that have been sequenced and assigned to genotypic lineages (Henningsen et al., 2024a).

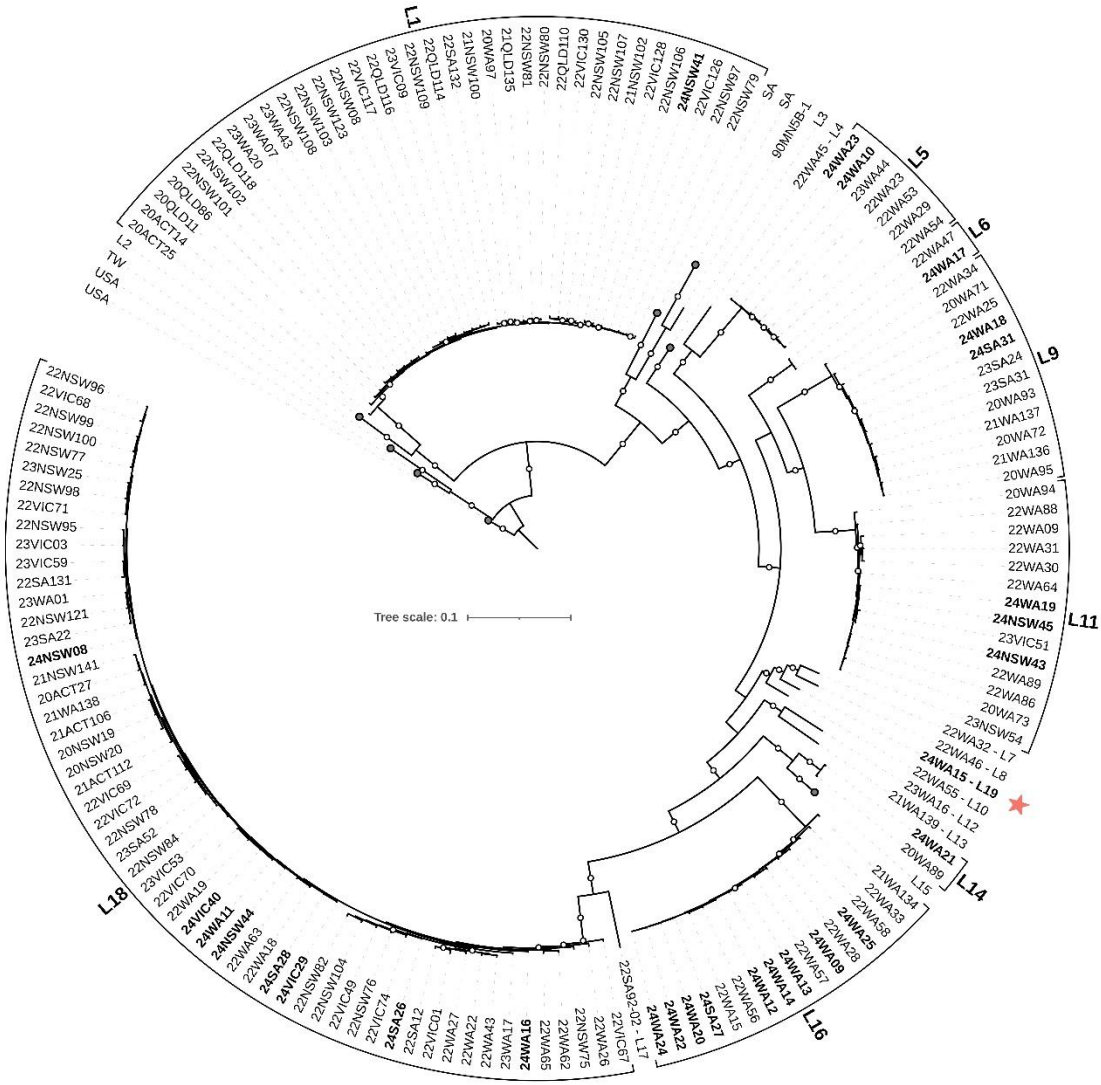


Fig 2. A Maximum likelihood phylogenetic tree of 380 *Pca* isolates worldwide. A midpoint-rooted maximum likelihood tree was constructed using 396,452 biallelic SNPs, with 500 bootstrap replicates. Bootstrap support values ≥ 80% are indicated by white circles. Isolates collected in 2024 are shown with bold text. Previously characterised Australian lineages (L1–L18) are shown with brackets. The newly described L19 is shown with a pink coloured star. Scale bar represents mean substitutions per site.

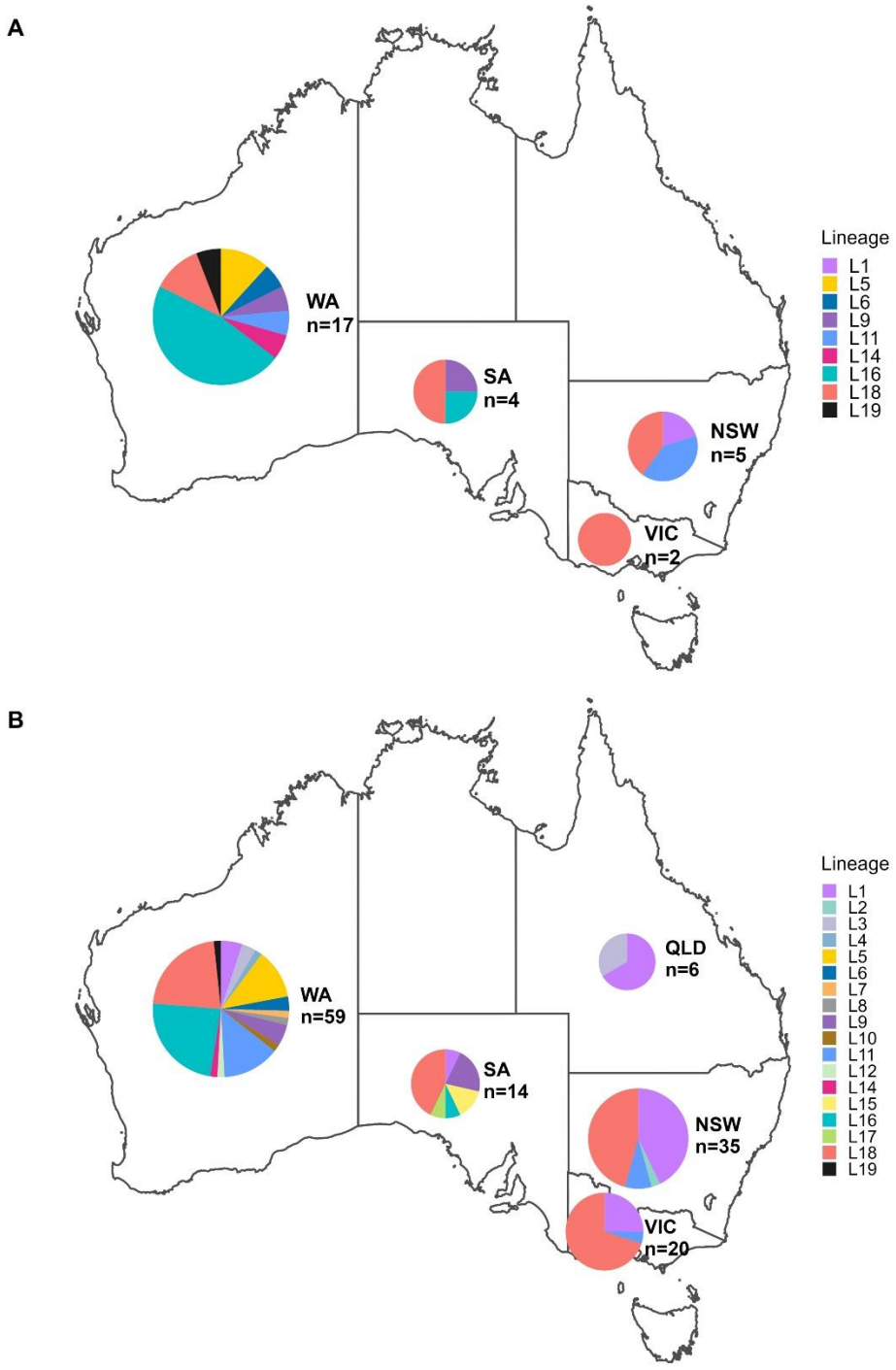
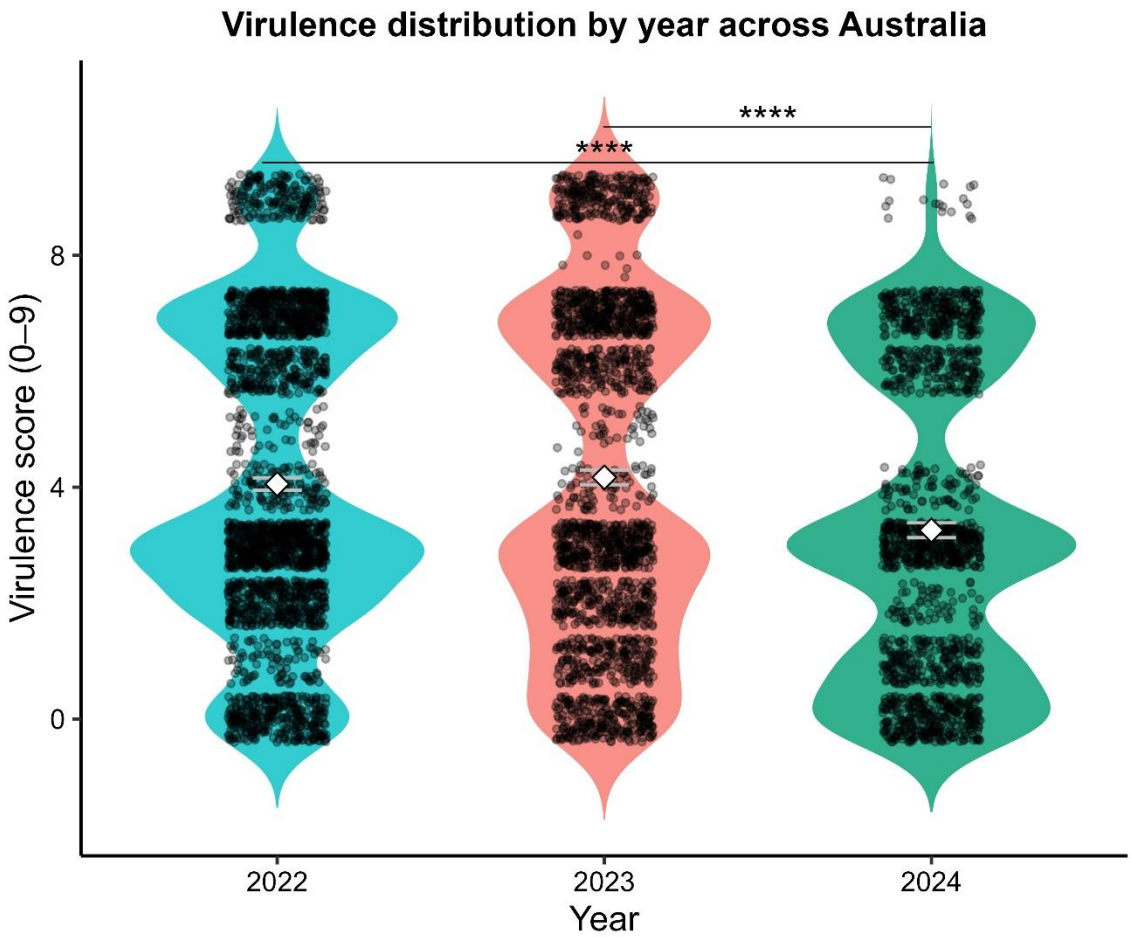
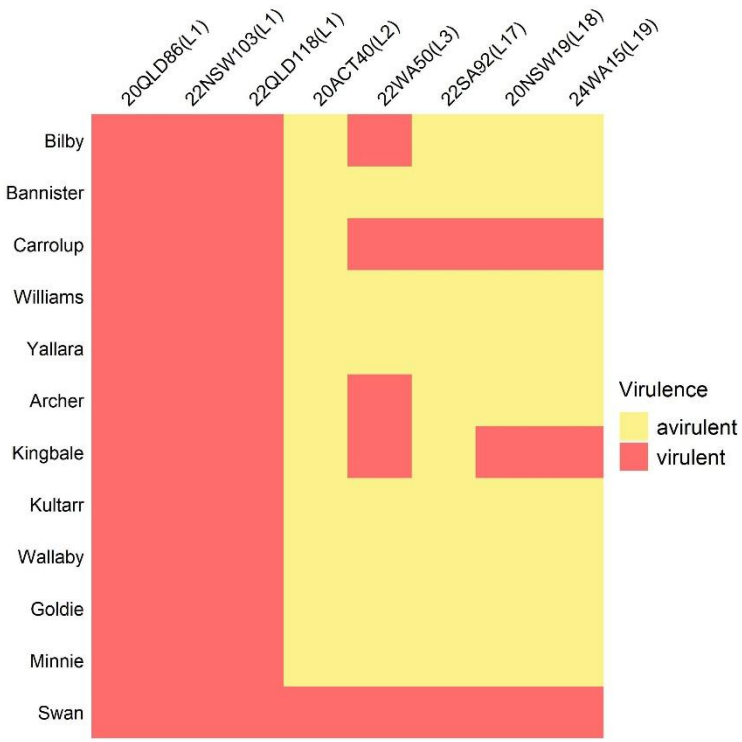


Fig. 3. Distribution and number of *Pca* isolates and lineages across Australia. **A.** Collection of *Pca* in 2024. **B.** Collection of *Pca* including samples from 2022 to 2024. Pie chart sizes reflect a logarithmic transformation of the total number of *Pca* isolates collected in their respective states. The pie chart for NSW includes isolates from both regions. Colors reflect genotypic lineage assignments.

Supplemental Information



Supplemental Figure 1. Distribution of infection scores of *Pca* isolates by year against oat differential lines. A box plot was drawn inside each violin plot to indicate median and inter-quartile range values. *p*-value was calculated using the Wilcoxon rank-sum test. **** indicates a highly significant difference ($p < 0.001$).



Supplemental Figure 2. Heatmap showing virulence profiles of *Pca* isolates representing L1, L2, L3, L17, L18, L19 (x-axis) on 11 oat commercial cultivars, plus Swan as susceptible control (y-axis). High infection scores indicating high virulence (susceptibility) are shown in red, and lower infection scores indicating avirulence (resistance) are shown in yellow.

Supplemental Table 1. Virulence scores of each *Pca* isolate collected in 2024 across the oat differential set.

Supplemental Table 2. Isolate metadata and sequencing information produced in this study.

Supplemental Table 3. Haplotype *k*-mer containment from *Pca* Illumina data.

Table 1. List of *Puccinia coronata* f. sp. *avenae* (*Pca*) isolates collected across Australia in 2024.

Isolate	City	State	10-letter race (pathotype)	Number of susceptible oat differential lines	Genotypic lineage number (L)
24NSW41	Gerogery	NSW	JBBRCBNFB-	10	L1
24WA10**	Quairading	WA	BJLQCCDCG-	10	L5
24WA23**	Meckering	WA	BJLQCCDCG-	10	L5
24WA17	Myrup	WA	BDBQCBDCQ-	8	L6
24WA18	Ravensthorpe	WA	BNBQMBNCG-	10	L9
24SA31	Williamstown	SA	BSBSMBDCB-	10	L9
24NSW43	Dhulura	NSW	BFLGMBDCG-	9	L11
24WA19	West River	WA	BPLGMBNCG-	11	L11
24NSW45	Wagga Wagga	NSW	BPLGMLDCG-	11	L11
24WA21	Moora	WA	BFPQMBDCG-	12	L14
24SA27	Hamley Bridge	SA	BDBGLBDCB-	5	L16
24WA13*	Perth	WA	BFBGMBDCG-	8	L16
24WA14*	Perth	WA	BFBGMBDCG-	8	L16
24WA22*	Calingiri	WA	BFBGMBDCG-	8	L16
24WA25*	Doodlakine	WA	BFBGMBDCG-	8	L16
24WA12*	Narrogin	WA	BFBGMBDCG-	8	L16
24WA24	Cunderdin	WA	BPBGMBDCG-	9	L16
24WA09	Gwambygine	WA	BPBGMBNCG-	10	L16
24WA20	Northam	WA	BTBGMBNCG-	11	L16
24NSW08	Boorowa	NSW	BFDGCHBCB-	9	L18
24WA11	Merridin	WA	BFDLBHDCG-	9	L18
24VIC40	Barnawartha	VIC	BFDQCCBCG-	9	L18
24WA16	Scaddan	WA	BFDQCHNCG-	14	L18
24SA26	Freeling	SA	BFDQLHBCB-	11	L18
24NSW44	Jugiong	NSW	BKDQBHLCB-	10	L18
24VIC29	Mitre	VIC	BKDQCHDCB-	11	L18
24SA28	Hahndorf	SA	BKDQMHDCB-	12	L18
24WA15	Lort River	WA	BKBGCHNCG-	11	L19
24SA30	Bordertown	SA	BFNGMHDCB-	11	-
24NSW39	Holbrook	NSW	BKDQMCBCB-	11	-

* and ** indicate isolates with identical race codes, while – indicates samples lacking Illumina sequencing data, and therefore a genotypic lineage could not be assigned.