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Pathogenic Specialization of *Puccinia hordei* Otth. in Australia, 1966–1990

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Abstract

One hundred and fifty-four isolates of the leaf rust pathogen (*Puccinia hordei*), collected from infected barley plants in Australia between 1966 and 1990, were typed to determine virulence with respect to the resistance genes *Rph1* to *Rph9*, *Rph12* (Triumph) and several uncharacterized resistance sources. The Australian cultivar, Prior, reacted differentially to the isolates examined, and is believed to possess a gene which is also present in addition to *Rph2* in Reka 1. Virulence and avirulence on Prior were designated P+ and P– respectively. Eleven distinct pathotypes (pt) were identified, with pt 243 P+ and 243 P– predominating in samples collected between 1966 and 1979. In the 1980*s*, pt 210 P+ was most commonly isolated from samples collected in Queensland and northern New South Wales, and although a range of different pathotypes was present in southern Australia, pt 200 P+ was most frequent in this region. Virulences to genes *Rph1, Rph2, Rph4, Rph5, Rph6*, *Rph8*, *Rph9* and *Rph12* have been detected, and only *Rph3* and *Rph7* are likely to be of value in protecting future Australian barleys from the disease.

Keywords: Barley, *Puccinia hordei*, pathotypes, differential cultivars, resistance.

Introduction

Genes conditioning resistance to leaf rust (*Puccinia hordei* Otth.) in barley are designated *Rph* genes (previously termed *Pa* genes) (Søgaard and von Wettstein-Knowles 1987). Twelve loci have been reported as governing resistance to leaf rust in barley (Roane and Starling 1967, 1970; Tan 1977*a*, 1977*b*, 1978; Feuerstein *et al.* 1990; Jin *et al.* 1993), although the chromosomal location and linkage relationships between some of these loci have not been determined. For example, the designation *Rph9* was given to a resistance gene in several Ethiopian barleys, but the relationship of this gene to genes *Rph1* to *Rph8* was not determined (Tan 1977*b*). Virulences to *Rph1* to *Rph9* and *Rph12* have been reported from *P. hordei* populations in the barley growing regions of the world (Ceoloni 1979; Reinhold and Sharp 1982; Manisterski *et al.* 1986; Jones 1988). Nevertheless, the occurrence of a virulence factor corresponding to the *Rph7* gene appears to be more restricted, being confined to North Africa, the Middle East, specifically Israel (Manisterski 1989), and recently, North America (Jin and Steffenson 1991).

Epidemics of leaf rust in barley have been reported in several Australian states during recent years with significant yield losses recorded in the popular malting

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barley cultivar, Grimmett, in two field trials (Cotterill *et al.* 1992*b*). Early work on pathogenic variability in Australia suggested the likely presence of two pathotypes of *P. hordei*. One was reported by Waterhouse (1952) as similar to a European pathotype, described earlier by d'Oliveira (1939), and the other differed from those reported by d'Oliveira (1939) and those by Mains and Martini (1932) in North America (Watson and Butler 1947). Luig (1985) reported that two pathotypes, UN14 and UN16 of Levine and Cherewick (1952), predominated in Australia prior to 1985.

The increased importance of leaf rust in recent years, resulting from an expansion in barley sowings, early and extended plantings of crops, and the continued use of susceptible cultivars (Cotterill *et al.* 1994), has led to greater emphasis being placed on the incorporation of resistance to leaf rust in Australian barley breeding programs. Because an understanding of the range of virulence in the *P. hordei* population is fundamental to these efforts, this study was undertaken to assess the pathogenic variability in *P. hordei* in Australia for the period 1966 to 1990.

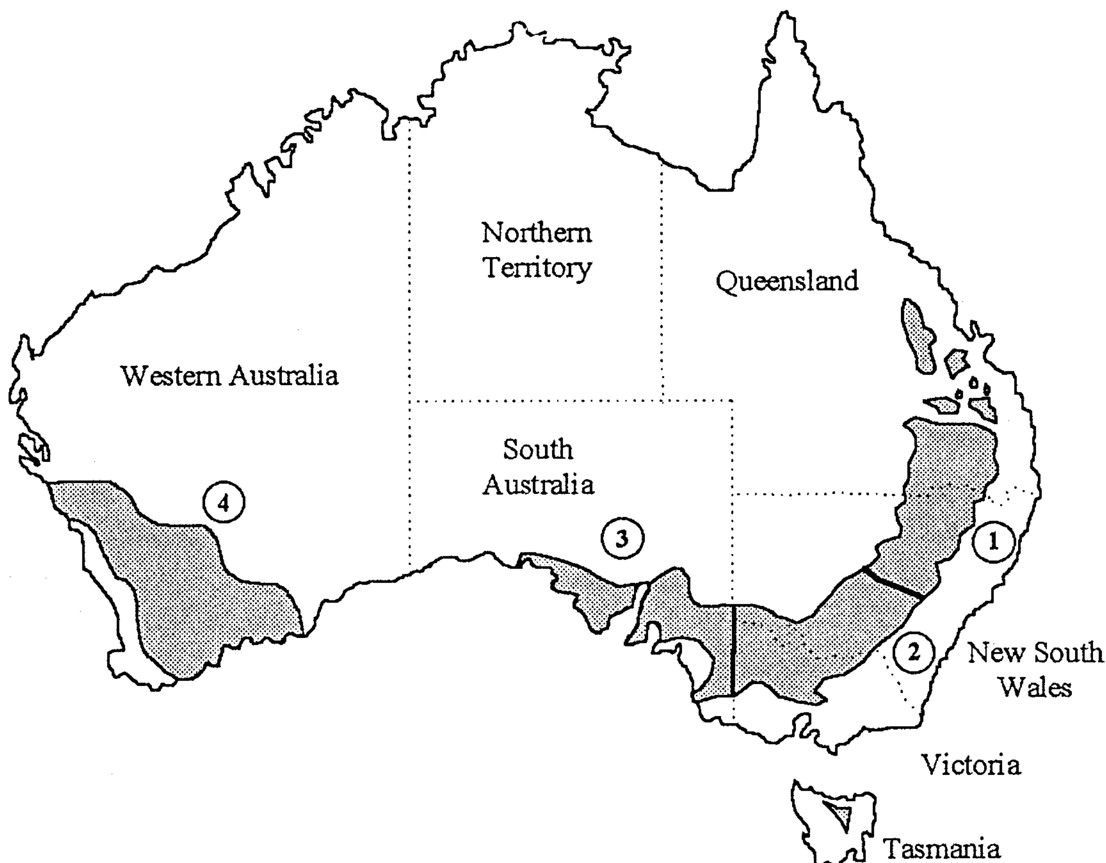

Fig. 1. The barley growing areas of Australia and the four climatically diverse regions.

Materials and Methods

Uredospores of *P. hordei* were collected from commercial crops or field trials in the cereal growing regions outlined in Fig. 1 between 1966 and January 1991 by various cooperators. At the Queensland Wheat Research Institute, Toowoomba, spores were increased on receipt of samples, or infected leaf pieces were dried and stored in liquid nitrogen. Uredospores were

increased on barley seedlings (cv. Gus) grown in a glasshouse, collected with a cyclone spore collector and stored in a desiccator above sulfuric acid (63% R.H.) for up to a few days before use.

Table 1. Set of barley differentials used to identify virulence factors in *P. hordei*, the genes involved, and their octal notation values

Differentials	Resistance gene(s)	Octal value
Sudan (Berg, Oderbrucker)[A]	*Rph1*	1
Peruvian (Reka 1 (*Rph2* +?), Ricardo (*Rph2* +?))	*Rph2*	2
Estate (Aim)	*Rph3*	4
Gold	*Rph4*	10
Magnif 104 (Quinn (+*Rph2*))	*Rph5*	20
Bolivia (+*Rph2*)	*Rph6*	40
Cebada Capa (Henry)	*Rph7*	100
Egypt 4	*Rph8*	200
Abyssinian	*Rph9*	400
Triumph	*Rph12*	4000
Prior	*RphP*	P+

[A] Additional differentials, listed in parentheses, were used for some genes.

A set of 10 differential barley cultivars (Jones and Clifford 1985), but with Estate replacing Ribari, plus eight additional cultivars including the Australian cultivar Prior, were used to type isolates of *P. hordei* (Table 1). Much of the work was undertaken before seed of lines possessing *Rph10* and *Rph11* (Feuerstein *et al.* 1990) was available and these lines were not used. Clumps of eight seedlings of each of three cultivars were grown in 10 cm diameter pots in a glasshouse with a temperature range of 15–25°C. When the second leaf was just visible, seedlings were inoculated with individual isolates of *P. hordei*. A small quantity of spores (*c.* 0·02 g) was mixed in *c.* 1 mL of a light mineral oil and sprayed onto the seedlings. Plants were misted with deionized water, placed in a dew simulation chamber at 17±1°C for 16 h (Clifford 1977) and then returned to the glasshouse.

Two to three days after initial eruption of uredia, plants were assessed for their disease response using a 0 to 4 scale (Parlevliet 1976), in which infection type (IT) 0 = flecks; 1 = minute pustules surrounded by mainly necrotic tissue; 2 = small pustules surrounded by chlorotic and/or necrotic tissue; 3 = pustules surrounded by chlorotic rings; 4 = pustules with little or no chlorosis; x = mesothetic (several IT on one leaf). IT 0 to 3− were considered resistant and those 3 to 4, susceptible. Where mixed reactions occurred, single uredial isolations were made and spores increased for re-typing. A pathotype code was established for each isolate of *P. hordei* using the octal notation system of Gilmour (1973) (Table 1).

At the Plant Breeding Institute, Cobbitty, samples of barley leaf rust periodically collected during the course of pathotype surveys of wheat rusts over the period 1966 to 1990, were stored in liquid nitrogen as dried leaf pieces bearing uredia or as dried uredospores. Uredospores were increased on cv. Gus if the inoculum in samples was insufficient to directly inoculate a differential set. Methods for inoculation and incubation of seedlings were similar to those outlined for wheat leaf rust by Park and Wellings (1992). Differentials used and procedures for assessing disease response and naming pathotypes were the same as those described above.

The identification of pathotypes was conducted in a series of experiments at both Institutes, and the identity of pathotypes was confirmed in most cases by repeat tests. Several cultures were exchanged for use in comparative tests, to ensure that the results obtained at each Institute were consistent.

Results

Cultivar Prior reacted differentially to the isolates examined, in being either susceptible (IT 4) or resistant (IT $0n/1^{-}$). All isolates which were found to be avirulent on Prior were also avirulent on Reka 1 producing an IT of $0n/1^{-}$. Reka 1 possesses *Rph2* and an unknown resistance gene which, from the results obtained, appears to be the same as that present in Prior. The only isolates found to be fully virulent on Reka 1 were those which combined virulence for Peruvian (*Rph2*) and Prior (pt 243 P+). Isolates avirulent for *Rph2* produced either IT 2 to $2^{+}/3$ or IT 0n to $0n/1^{-}$ on Reka 1, depending on whether they were virulent or avirulent, respectively, on Prior. To indicate virulence or avirulence on Prior, pathotype codes were qualified by P+ or P−respectively.

Only limited isolates of *P. hordei* were available for typing for the period 1966 to 1979. These isolates were collected from widely separated sites, and except for two isolates, were typed as pt 243 P+ or 243 P− (Tables 2 and 3). Pt 243 P+ and 243 P− were apparently the main pathotypes involved in the Queensland epidemic of 1978.

Pathotype 210 P+ (virulent on *Rph4* and *Rph8*) was first recorded in 1978 in region 1. An epidemic again occurred in Queensland in 1983 and 6 of 21 isolates from Queensland were of pt 210 P+. This pathotype predominated in samples collected through to 1990 and was involved in epidemics in region 1 in 1984 and 1988.

Pathotype 200 P+ was initially recorded from the 1983 epidemic in Queensland. Subsequently it became widespread but at a relatively low level except in 1990 in New South Wales (N.S.W.) and Victoria.

Discussion

Eleven distinct pathotypes were identified from the samples examined in the present study (Table 2). Earlier studies by Waterhouse (1927, 1952) and Luig (1985) indicated a lower level of pathogenic diversity in the Australian barley leaf rust population. Virulence to *Rph4* and Reka 1 (*Rph2* + ?) was detected by Waterhouse (1927). Although it is possible that his isolate could have been pt 210 P+, virulence combinations cannot be determined, since Waterhouse (1927) did not have available all of the differential barleys currently in use. Watson and Butler (1947) reported virulence to *Rph1* and *Rph8* and avirulence to Reka 1; this pathotype was possibly pt 201. However, the virulence combinations, *Rph2, Rph4* and *Rph8*; *Rph1, Rph2, Rph4* and *Rph8* (UN pathotypes 16 and 14, respectively; Levine and Cherewick 1952) reported by Luig (1985) to predominate prior to 1985, were not detected in the present study. This could be due to inadequate sampling prior to 1985.

Detailed studies on pathogenic variation in *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn. in Australia have demonstrated that new pathotypes arise primarily from existing pathotypes by single- (or rarely double-) step mutations

Table 2. Frequency of isolates of pathotypes of *P. hordei* identified, classified by year, 1966–1990

Year	Pathotype: 20 P+	200 P+	201 P+	210 P+	211 P+	220 P+	230 P+	243 P+	243 P−	253 P−	4610 P+	Total
1966	—	—	—	—	—	—	—	1	1	—	—	2
1967	—	—	—	—	—	—	—	2	1	—	—	3
1969	—	—	—	—	—	—	—	—	1	—	—	1
1974	—	—	—	—	—	—	—	1	1	—	—	2
1976	—	—	—	—	—	—	—	1	1	1	—	3
1978	—	—	—	1	—	—	—	4	5	—	—	10
1979	—	—	—	—	—	—	—	2	—	—	—	2
1983	3	1	—	6	—	4	—	2	7	—	—	23
1984	—	—	—	4	—	—	—	—	—	—	—	4
1985	—	2	—	2	—	—	—	1	1	—	—	6
1986	—	1	—	6	—	—	—	1	—	—	—	8
1987	—	—	—	1	—	1	—	—	—	—	—	2
1988	—	1	—	16	1	—	—	3	—	1	—	22
1989	1	5	1	4	—	—	—	—	—	—	—	11
1990	1	13	—	24	2	2	2	3	3	4	1	55
Total	5	23	1	64	3	7	2	21	21	6	1	154

Table 3. Frequency of isolates of pathotypes of *P. hordei* identified, classified by state and region, 1966–1990

State	Region	Pathotype: 20 P+	200 P+	201 P+	210 P+	211 P+	220 P+	230 P+	243 P+	243 P−	253 P−	4610 P+
Qld	1	3	3	—	45	1	3	—	10	10	1	—
N.S.W.-north	1	—	8	—	10	1	1	—	2	1	2	—
N.S.W.-south	2	—	6	—	4	—	3	—	4	3	—	—
Vic.	2	1	5	1	3	1	—	1	2	2	—	—
Tas.	2	—	—	—	1	—	—	—	—	1	2	1
S.A.	3	1	1	—	—	—	—	—	—	—	—	—
W.A.	4	—	—	—	1	—	—	1	1	3	1	—
Unknown		—	—	—	—	—	—	—	2	1	—	—
Total		5	23	1	64	3	7	2	21	21	6	1

of loci governing virulence/avirulence. Variation may also arise less frequently through somatic recombination or introduction from geographic regions outside Australasia (Luig 1977). The occurrence of a group of *P. hordei* pathotypes during the early 1980*s* which were distinct from pt 243 (Table 1) may have resulted from the introduction of an exotic pathotype into Australia, and subsequent development of related pathotypes through single-step mutations. For example, pts 201, 210 and 220 could have been derived from pt 200 through the acquisition of virulence for *Rph1*, *Rph4* and *Rph5* respectively. Similarly, pt 253 P− could have been a single-step mutant derivative of pt 243 P−. Alternatively, some of the pathotypes could have originated from sexual recombination. Infected plants

of the alternate host, *Ornithogalum umbellatum* L., were located on the Yorke Peninsula, S.A., and six pathotypes of *P. hordei* were identified from seven isolates derived from aeciospores (Wallwork *et al.* 1992). One of the pathotypes (200 P+) was virulent on Schooner, a popular barley in South Australia, and one isolate of this pathotype was also detected in South Australia in the present study.

The reason for the predominance of pt 210 P+ in samples collected from region 1 is probably related to the popularity of the cultivar Grimmett, which was released in 1982. In 1983, plantings of this cultivar accounted for approximately 60% of total Queensland barley plantings, with the balance sown largely to Clipper, Galleon, Corvette and to a lesser extent, Lara. These four cultivars are susceptible to many pathotypes of *P. hordei*, whereas Grimmett is susceptible to only two (pt 210 P+ and 253 P−) of the 11 pathotypes tested by Cotterill *et al.* (1994), suggesting that it possesses the *Rph4* resistance gene. It is therefore quite likely that in the leaf rust epidemic of 1983, populations of several pathotypes known to be present prior to 1983 (e.g. 210 P+, 243 P−, 243 P+) increased and pathotypes which had not been recorded previously were also detected. In 1984, Grimmett accounted for over 80% of barley plantings in Queensland and continued to do so until 1990. Therefore it is likely that in the epidemic of 1984 the predominance of Grimmett favoured pt 210 P+, as none of the other pathotypes detected in 1983 were virulent on this cultivar.

A greater range of cultivars is grown in regions 2 and 3 than in region 1, and the majority of these cultivars is susceptible to most Australian pathotypes of *P. hordei* (Cotterill *et al.* 1994), whereas only two pathotypes are virulent on Grimmett. Hence, the selective pressure on specific pathotypes of *P. hordei* was less in regions 2 and 3 than in region 1. Despite this, it is clear that pt 210 P+ was detected more frequently in regions 2 and 3 during 1990, and it was also detected in Western Australia (region 4) in that year. The occurrence of pt 210 P+ in regions 1, 2 and 3 during 1990 could have been related to the growing of Skiff (a cultivar released in 1988), since this cultivar is susceptible only to pt 210 P+ (Cotterill *et al.* 1994). Similarly, in Tasmania, the growing of Shannon, which like Grimmett is resistant to all but pt 210 P+ and 253 P− could explain the occurrence of these two pathotypes, and the occurrence of pt 4610 P+ in this state (Cotterill *et al.* 1992*a*) probably relates to the growing of Franklin, released in 1989.

Cotterill *et al.* (1994) found that of 11 pathotypes used to test 38 commercial Australian barleys, four were avirulent to Cutter, Ketch, Prior, Schooner and Weeah. The results presented here strongly suggest that the gene shared by these cultivars is the same as the uncharacterized resistance gene present (in addition to *Rph2*) in the cultivar Reka 1. Given that the Australian cultivars appear to possess only one resistance gene effective against certain Australian pathotypes of *P. hordei*, these cultivars were considered more suited than Reka 1 for the Australian pathogenicity survey. Octal pathotype numbers were therefore qualified by the addition of P+ or P− to indicate virulence or avirulence, respectively, on cultivar Prior, which was one of the most widely grown barley cultivars in Australia for many years. As an interim measure, this system will be adopted for future typing of *P. hordei* in Australia, until the gene(s) involved has been fully characterized by genetic studies. The previously reported pathotype 1610

(Cotterill *et al.* 1992*a*) is classified as 4610 P+ using this system (the designation of the Triumph resistance as *Rph12* (Jin *et al.* 1993) now makes this pathotype 4610 on the octal scale).

We have shown that within the mainland Australian population of *P. hordei* there is virulence to all of the designated genes except *Rph3, Rph7, Rph9* and *Rph12*. Virulence to *Rph9* and *Rph12* has been detected in Tasmania (Cotterill *et al.* 1992*a*) and in South Australia and southern N.S.W. during 1993 (R.F. Park, unpublished data). The release of two cultivars possessing *Rph12*, Franklin in Tasmania and Tallon in Queensland, has been beneficial in the short term, but it is likely that *P. hordei* virulent on *Rph12* will increase in frequency and pose a threat to both cultivars. For this reason the *Rph3* and *Rph7* genes are being utilized in the Queensland barley breeding program and slow-rusting resistance is also being evaluated and exploited in breeding.

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