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Long Term Surveys of Pathogen Populations Underpin Sustained Control of the Rust Diseases of Wheat in Australia

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Abstract

The wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici* (*Pgt*) and wheat leaf rust pathogen *P. triticina* have been present in Australia at least since European colonisation. The stripe rust pathogen of wheat, *P. striiformis* f. sp. *tritici* (*Pst*), was first detected in 1979. Surveys of pathogenic variability in *Pgt* and P. *triticina* began in 1921/22, and of *Pst* in 1979, and have continued annually, uninterrupted ever since. These surveys involve the identification of races (pathotypes) in greenhouse assays, using wheat genotypes ("differentials") carrying different resistance genes. Virulence determinations have targeted principally all stage ("seedling") resistance genes, and only rarely adult plant resistance because of the technical difficulties of working with adult plants under controlled conditions. Data compiled since surveys for each pathogen began strongly implicate periodic introduction of exotic isolates, single-step mutation, and more rarely somatic hybridisation, as the major processes generating genetic diversity. The surveys have also provided clear evidence of migration of rust isolates throughout Australian cereal growing regions, with many examples of inoculum exchange between the eastern and western cereal belts, principally in a west to east direction. The long term surveys of wheat rust pathogens in Australia have provided both information and pathogen isolates that have underpinned rust control efforts, from gene discovery to post-release management of resistance resources. Increasingly, information on the pathogenicity of rust isolates is being complemented by estimates of genetic diversity, using selectively neutral markers to gain refined insight into the evolution and maintenance of virulence, migration pathways, and periodic long-distance migration events.

Keywords

Biosecurity; genetics; *Puccinia*; resistance; surveillance; *Triticum*

Introduction

The demonstration of pathogenic variation in the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici*; *Pgt*) almost 100 years ago by Stakman and Piemeisel (1917), and later in the wheat leaf rust pathogen (*Puccinia triticina*; Mains and Jackson, 1921) and the wheat stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*, *Pst*; Gassner and Straib, 1932), were crucial steps in the effort to develop wheat varieties with durable genetic resistance to these important plant pathogens. These early studies identified wheat genotypes ("differentials") that varied in response to rust isolates, and the pathogen variants identified on these standard differentials were referred to as races. Later studies showed that some races could be further subdivided based on pathogenicity on additional differential genotypes, and these sub-types within races

were referred to as strains or pathotypes. Race (pathotype) surveys of the three wheat rust pathogens, which involve greenhouse testing of rust isolates on seedlings of differential genotypes, have since been undertaken in many regions of the world.

In Australia, surveys of the wheat rust pathogens *Pgt* and *P. triticina* were initiated by Professor W. L. Waterhouse at the University of Sydney in 1921/22, and have continued uninterrupted since. Surveys of *Pst* began in 1979 following its first detection in Australia (Wellings, 2007). Professor Waterhouse was President of the Royal Society of New South Wales in 1937, and his Presidential Address published in the Society journal in 1938 was titled "Some aspects of problems in breeding for rust resistance in cereals" (Waterhouse, 1938). In drawing attention to the importance of rust diseases in wheat, it was stated that "the ravages of rust in New South Wales have caused losses amounting to an average amount of £250,000 per annum during the past twenty years". Noting the discovery by Biffen of Mendelian inheritance of resistance to rust in wheat, Waterhouse (1938) further discussed the nature of resistance to rust in wheat, describing three main forms of resistance shown by plants to rust. A summary of the results of pathotype surveys for *Pgt*, *P. triticina*, and several other rust pathogens of oat and barley from 1922 to the end of 1937 were then provided.

Annual surveys of pathogenic variability in the three wheat rust pathogens have continued uninterrupted since 1921/22 (*Pgt* and *P. triticina*) and 1979 (*Pst*), at the University of Sydney. The information generated from this work has been crucial to rust control efforts based on genetics, which have delivered substantial benefits to the Australian economy.

Wheat Rust Pathotype Surveys in Australia

A fascinating feature of some rust fungi is heteroecism; a requirement by some species for two unrelated hosts for completion of the full life cycle. *Pgt* and *Pst* require both wheat (a monocot) and certain species of *Mahonia*, *Berberis*, or *Mahonia* x *Berberis* hybrids (dicots) to complete their full life cycles, while *P. triticina* requires wheat and either *Thalictrum speciosissimum* or *Isopyrum fumaroides* (both dicots). In all three pathogens, sexual recombination occurs on the dicot host, which is often referred to as the alternate host. The alternate hosts of all three rust pathogens are rare or absent in Australia, and it is generally believed that sexual recombination is similarly rare or absent here (Watson and Luig, 1958; Wellings, unpublished). Data compiled since surveys for each pathogen began strongly implicate periodic introduction of exotic isolates, single-step mutation, and more rarely somatic hybridisation, as the major processes generating genetic diversity. The surveys have also provided clear evidence of migration of rust isolates throughout Australian cereal growing regions, with many examples of inoculum exchange between the eastern and western cereal belts, principally in a west to east direction (Park et al., 1995).

Rust resistance gene and pathotype nomenclature

More than 204 resistance genes/ alleles have been characterised in wheat to date (Park, 2016). Because of the relatively large chromosome number in hexaploid wheat and the large number of resistance genes already known, new genes are usually mapped to a chromosome arm before being permanently named. Linkage studies and tests of allelism may then be necessary to determine if the locus is distinctive. Once

distinctiveness is proven, the locus is numbered using the designation *Sr* for stem rust, *Lr* for leaf rust, and *Yr* for stripe rust.

Many different systems have been devised to name rust pathotypes. The systems used in Australia were outlined by Park (2008). Pathotypes of *Pgt* are identified following the system described by Watson and Luig (1963) in which six genotypes permit a standard race designation (Stakman *at al.,* 1962), and an additional 11 wheats and two triticales numbered from 1 to 13 (the Australian supplemental differentials) allow further characterisation (Park, 2008). Wheat leaf rust pathotypes are identified using four genotypes that permit a standard race designation (Johnston and Browder 1966), and an additional 11 wheats used as Australian supplementary differentials. Pathotypes of *Pst* are identified using an International differential series and a European series, with a number assigned to each series by the addition of decanery values corresponding to each differential rendered susceptible. The second number is preceded by the letter E to indicate the European series. In Australasia, the pathotype formula is followed by A- or A+ to indicate avirulence or virulence, respectively, for a distinctive resistance present in a selection of the Australian cultivar, Avocet (Wellings *et al.,* 1988) *Puccinia graminis* f. sp. *tritici.*

The population structure of *Pgt* over the past 93 years was strongly influenced by exotic introductions in 1925 (standard race 126), 1954 (standard race 21), and 1969 (standard races 194 and 326) (Table 1), subsequent random mutations to virulence, and selection of genotypes with virulence matching resistance genes in cultivars.

Prior to the detection of race 126 in Western Australia in 1925, Waterhouse (1952) identified six races, differentiated by their virulence/ avirulence on 12 differential wheat genotypes used in previous in studies in the USA by Stakman and Piemeisel (1917). Studies of the abilities of these six races to infect a wide range of wheat genotypes suggested they belonged to two race groups, one comprising races 43, 44 and 54, and one comprising races 45, 46 and 55 (Waterhouse, 1938). Race 126 spread to eastern Australia and by 1929 had all but superseded the six races detected previously, presumably due to increased aggressiveness (Waterhouse, 1952). The original race 126 along with several derivative pathotypes predominated until 1954, when pathotype 21-0 was first detected, believed to have originated either from *Berberis* in Tasmania (Watson, 1958) or from Africa (Luig, 1977). The frequency of this pathotype increased rapidly in eastern Australia over the next few years, and that of the 126- group declined. Over 50 new pathotypes, all considered to have arisen via step-wise mutations tracing back to pathotype (pt.) 21-0, were detected during the 1950s and 1960s (Luig and Watson, 1970).

Pathotype 34-2,11 was first detected in northern New South Wales in 1957. It combined certain pathogenic (Watson 1981) and isozymic (Burdon et al., 1982) features of both the 126- and the 21- groups, and on this basis was regarded to have arisen via somatic hybridisation between the two groups.

In 1969, two distinct pathotypes (194-1,2,3,5,6 and 326-1,2,3,5,6) were detected for the first time. Both were initially identified from six samples collected during 1969 from New South Wales, Victoria and South Australia, and in 1970, in samples collected from Western Australia (Luig and Watson, 1970). Comparative studies of a range of features suggested that both originated from central Africa, possibly being transported to Australia by high altitude winds across the Indian Ocean (Watson and de Sousa, 1982). The first pathogenic change in these

pathotypes was the development of pt 343-1,2,3,5,6, regarded as a mutant of pt 326-1,2,3,5,6 with added virulence for *Sr5* and detected in 1973 (Watson, 1981). Several other subsequent changes were detected, and members of this group are now well established in Australian wheat growing regions.

Pathotypes of *Pgt* detected in Australia during the past 10 years trace back to pathotypes 21-0, 194-1,2,3,5,6 or 326-1,2,3,5,6 (R.F. Park, unpublished).

Puccinia triticina

The origins and evolutionary relationships between pathotypes of *P. triticina* detected prior to 1980 are not as well understood. Pathogenic variation in *P. triticina* has been monitored since 1926, when Waterhouse (1929) reported two pathotypes that could be differentiated on the Australian wheat cultivar (cv.) Thew (*Lr20*). New virulences were subsequently detected for resistance genes including *Lr3a*, *Lr3ka*, *Lr14a*, *Lr15*, *Lr23* and the complementary genes *Lr27+Lr31*.

Critical surveys of the leaf rust population in Australia between 1980 and 2013 provided strong evidence of five exotic incursions (Table 1), each of which has acted as a founding isolate and given rise to clonal lineages through sequential acquisition of virulence to single resistance genes: pathotype (pt.) 53-1,(6),(7),10,11 (first detected in 1981); pt. 104-2,3,(6),(7),11 (1984); 76-1,3,5,10,12 (1996); 10-1,3,9,10,12 (2006); 76-3,5,9,10 +Lr37 (2006). A sixth lineage, derived from somatic hybridisation between isolates related to pts 53-1,(6),(7),10,11 and 104-2,3,(6),(7),11, was first detected in northern NSW in 1990 (pt. 64-(6),(7),(10),11; Park *et al.*, 1999).

The incursions of five exotic *P. triticina* isolates during this 33 year period is of particular interest given that only one other exotic incursion of a wheat attacking rust was detected during that time (*Pst* in 2002; Table 1). The origin of each, the means by which they were introduced, and just why so many incursions of *P. triticina* have occurred, remain unknown. Also of interest is that while a local mutation to virulence for *Lr13* has not been detected, four of the five incursions carry virulence for this gene (viz. pts 53-1,(6),(7),10,11; 76-1,3,5,10,12; 10-1,3,9,10,12; 76-3,5,9,10 +Lr37). Gene *Lr13* is common in Australian wheat cultivars, and prior to the detection of 53-1,(6),(7),10,11 in 1981, was effective. Following 1981, combinations of *Lr13* with genes such as *Lr1* (e.g. cv. AGT Katana, Arnhem, Diamondbird, Hartog, Kukri), *Lr17b* (e.g. cv. Declic, Lawson, Paterson), *Lr24* (e.g. cv. Naparoo), *Lr26* (e.g. cv. Grebe), and *Lr37* (e.g. cv. Axe, Crusader) remained effective but eventually succumbed either as a result of the subsequent three incursions and/or mutational derivatives of the four *Lr13*-virulent pathotypes.

Puccinia striiformis f. sp. *tritici*

Stripe rust of wheat was first detected in Australia in 1979 (O'Brien et al., 1980). The disease was first detected in Victoria and spread rapidly throughout most of the eastern Australian wheat belt. The pathotype detected in 1979 was identified as 104 E137 A-, and was considered to have been transported to Australia on contaminated clothing, likely from Europe (Wellings, 2007; Table 1). From 1979 to 2006, at least 20 new *Pst* pathotypes were identified in Australia, each presumed single-step mutant pathotypes derived sequentially from the original pathotype detected in 1979 (Wellings, 2007).

Stripe rust was not recorded in Western Australia until August 2002, when it was detected in the Newdegate Shire (Wellings et al., 2003). Analyses demonstrated the

presence of a single pathotype (pt. 134 E16 A+) that was distinct from eastern Australian *Pst* pathotypes not only in pathogenicity but also in AFLP phenotype, indicating a likely exotic origin (Wellings et al., 2003; Table 1). The new pathotype was subsequently detected in eastern Australia (southern New South Wales and South Australia) in September 2003, and has dominated the *Pst* population in all Australian wheat regions surveyed since then (Wellings, 2007). While estimates of the cost of fungicidal control in 2003 were about AUD $43 million, a more severe epidemic developed in 2004 and an estimated AUD $90 million was spent on chemical control (CR Wellings, unpublished data).

Detailed studies of pathotype 134 E16 A+ demonstrated that its virulence profile on specific all stage resistance genes did not pose any greater threat to Australian wheat cultivars. Despite this, many cultivars were noticeably more susceptible to this pathotype at later adult plant growth stages (Wellings and Bariana, 2005). It is generally accepted that pt. 134 E16 A+ is more aggressive than pathotypes related to the 1979 incursion. While studies overseas showed that increased aggressiveness in *Pst* was at least in part attributed to adaptation to higher temperatures (Milus et al., 2006), Australian studies of pt. 134 E16 A+ under controlled conditions failed to show this and suggested that its increased aggressiveness is due to other factors (Loladze et al., 2014). Circumstantial evidence has suggested that this pathotype carries virulence for an uncharacterized APR gene common in current Australian wheat cultivars.

Pathotype Surveys and Rust Control

To have maximum impact in disease control, surveys of pathogenic variability in rust pathogens must be closely integrated with the development and management of new wheat cultivars. Where this has been practiced, surveys have provided both information and pathogen isolates that have underpinned rust control efforts, from gene discovery to post-release management of resistance resources. Information generated by pathotype surveys has been used to devise breeding strategies, inform selection of the most relevant isolates for use in screening and breeding, define the distribution of virulence and virulence combinations, allow predictions of the effectiveness / ineffectiveness of resistance genes, and issue advance warning to growers by identifying new pathotypes that overcome the resistance of cultivars before they reach levels likely to cause significant economic damage.

Rust resistance genes in wheat

In 1938, the year Waterhouse's Presidential address to the Royal Society was published, the first stem rust resistant wheat was released in Australia - cv. Eureka. It is now known that Eureka is protected from *Pgt* by the single resistance gene *Sr6*, located on wheat chromosome 2DS. Since then, many loci conferring resistance to the three rust pathogens of wheat have been catalogued: 62 genes/ alleles at 55 loci conferring resistance to *Pgt* (*Sr2* – *Sr58*, the symbols *Sr1*, *Sr3*, *Sr4* and *Sr9c* were abandoned due to a lack of reference stocks or duplication with previously described loci); 73 resistance genes/ alleles conferring resistance to *P. triticina* (*Lr1* to *Lr73*; the symbols *Lr4*, *Lr5*, *Lr6*, *Lr7*, *Lr8*, *Lr40*, *Lr41* and *Lr43* were abandoned due to a lack of reference stocks or duplication with previously described loci); and 69 genes/ alleles at 65 loci (*Yr1* – *Yr67*; *Yr26* is considered to be synonymous with *Yr24*) conferring resistance to *Pst* (Park, 2016).

While most rust resistance genes identified in wheat confer resistance at all growth stages (often referred to as seedling or major genes), some confer resistance only at post-seedling growth stages (often referred to as adult plant resistance (APR) genes). Genes conferring all stage resistance have a major effect on the resistance phenotype when challenged with an avirulent isolate, and consequently have also been referred to as "major genes". APR genes on the other hand can have either major, or more frequently minor, effects on the resistance phenotype, and are accordingly often referred to as "minor genes".

Establishing the genetic relationships between loci conferring rust resistance and their effectiveness has been crucial in gene deployment, ensuring optimal protection of crops from rust infection. This research has relied on the use of rust isolates with defined virulence/ avirulence attributes, which have come from pathotype surveys. One important outcome from this research has been the demonstration that some loci comprise alleles that cannot be combined in a homozygous state by hybridisation. Three of 55 loci conferring resistance to *Pgt* are known to comprise allelic series, *viz*. *Sr7* (alleles *Sr7a* and *Sr7b*), *Sr8* (*Sr8a*, *Sr8b*), and *Sr9* (*Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9f*, *Sr9g*, *Sr9h*) (Park, 2016), three loci conferring resistance to *Pst* comprise multiple alleles (*viz*. three alleles at the *Yr3* locus, two at the *Yr4* locus, and recently *Yr5* and *Yr7* were shown to be allelic), and allelic variation has been recorded at four loci conferring resistance to *P. triticina* (*Lr2*, *Lr3*, *Lr17* and *Lr22*).

Long term studies of pathogenic variability in Australia have also identified several resistance genes for which virulence has not been detected, including three pleiotropic loci that confer APR to all three rust pathogens and to the powdery mildew pathogen *Blumeria graminis* f. sp. *tritici*: *Lr34/Yr18/Sr57/Pm38* (Spielmeyer et al., 2005); *Lr46 / Yr29 / Sr58 / Pm39* (Lillemo et al., 2008); *Lr67/Yr46/Sr55/Pm46* (Herrera-Foessel et al., 2014). All three loci are associated with the morphological trait leaf tip necrosis. A fourth slow rusting APR locus, *Sr2/Yr30*, confers resistance to stem rust and stripe rust and is completely associated with the morphological trait pseudo black chaff (Singh et al., 2005). With the exception of the *Lr67/Yr46/Sr55/Pm46* locus, all have been used widely in commercial wheat varieties over many years and to date no cases of virulence have been reported from pathogenicity surveys.

The value of rust resistance breeding as exemplified by stem rust

Resistance genes have been used to great effect in protecting Australian wheat crops from rust. In estimating the current and potential costs of diseases of wheat in Australia, Murray and Brennan (2009) calculated that rust control through resistance breeding, cultural methods and chemical intervention saved growers more than $1.5 billion per year, of which about 67% ($1 billion) was due to resistance breeding. The biggest impact of resistance breeding in control was estimated for stem rust, accounting about 93% of the overall value of control estimated at $470 million per year.

Much of the success in controlling stem rust in Australia, especially in the rust-prone region of north eastern Australia, has been based on exploiting knowledge of pathogen variation in the development of new wheat cultivars. From 1919 to 1938, wheat cultivars were susceptible to the six races detected by Waterhouse (1938). Attempts to breed for resistance to these six races involved crosses between two wheats bred by William Farrer, Canberra and Thew, each carrying different

stem rust resistance genes. A line selected from this cross yielded cv. Euston, which combined the resistances of the two parents and was resistant to the six rust races. Euston was however rendered susceptible by the arrival of race 126 in 1925 and was never grown commercially (Watson and Butler, 1984).

The release of Eureka in 1938 heralded a second phase in stem rust resistance breeding (1938 to 1964) during which cultivars with single genes for resistance were released and new pathotypes with corresponding virulence were detected. Following its release in 1938, cv. Eureka increased in popularity and by 1945 occupied about 18% of the wheat area in northern New South Wales and Queensland (Watson and Luig, 1963). Virulence for *Sr6* was first detected in 1942, and its frequency in the *Pgt* population in this region increased as the area sown to Eureka increased (Watson and Luig, 1963). Examples of other resistance genes that succumbed to presumed mutations in *Pgt* over subsequent years include *Sr11* (cv. Gabo, released in 1942, virulence first detected in 1948), *Sr17* (cv. Warigo, released in 1943, virulence first detected in 1959), and *Sr9b* (cv. Dowerin, released in 1948, virulence first detected in 1960) (Park 2007).

The loss of resistance in cultivars with single gene resistance during this time led to the concept of combining resistance genes to bestow greater durability, proposed by researchers more than 50 years ago (e.g. Watson and Singh, 1952). From 1965 onwards, cultivars with multiple resistance genes were deployed in many regions of Australia, significantly reducing yield losses due to stem rust epidemics. During this third phase and until now, cultivars were protected by resistance genes *Sr2*, *Sr9g*, *Sr12*, *Sr13*, *Sr17*, *Sr22*, *Sr24*, *Sr26*, *Sr30*, *Sr36*, and *Sr38*, singly or more commonly in combinations.

Overall inoculum levels and pathotype diversity in *Pgt* have declined in all wheat-growing regions since the mid-1970s, likely as a consequence of the release of cultivars with such resistance gene combinations. For example, while some 40 pathotypes of *Pgt* were identified in 1973, only 14 pathotypes were detected for the 10 year period 2003–2013 (Zwer *et al.*, 1992; R.F. Park, unpublished).

Concluding Comments, Future Challenges and Directions

Exotic rust threats

The frequency of exotic incursions of wheat rust isolates into Australia has increased since the first such incursion was detected in 1925 (Table 1), presumably due to inadvertent human-mediated transport of rust spores associated with increased and more rapid international travel. Efforts to breed for resistance to rust have been successful in pre-empting local mutations to virulence (McIntosh and Brown, 1997), but less successful in dealing with exotic incursions of rust isolates due to the inability to anticipate when these will occur, from where they will come, and consequently the virulence of any isolates that are introduced.

The only wheat attacking rust pathogen not present in Australia is leaf rust of durum wheat caused by a variant of *P. recondita* that has *Anchusa* as its alternate host (Anikster et al. 1997). While this pathogen is considered a serious exotic threat, new pathotypes of the three rust pathogens already in Australia also pose serious threats, as clearly shown by the impact of the incursions of new pathotypes of *Pgt* in 1925, 1954 and 1969, and of *Pst* in 2002. Of particular significance in this regard is "Ug99", detected in Africa in 1999 following the observation of severe stem rust infection in wheat nurseries in Uganda in

1998. Greenhouse assays of a single sample of stem rust collected from these nurseries (accession "Ug99") in South Africa identified the presence of a *Pgt* pathotype that has since become known widely as "Ug99" and has the North American race designation TTKSK (Pretorius et al., 2000; Jin et al., 2009). This pathotype overcomes many of the resistance genes that protect wheat cultivars from stem rust, including *Sr31*, a gene for which virulence had not been previously detected. Analyses carried out on samples of stem rusted wheat collected from across a wide area have since shown that pathotype TTKSK is a member of a family of closely related pathotypes that is now known as the "Ug99" lineage. In addition to Uganda, one or more of these pathotypes are present in Eritrea, Ethiopia, Iran, Kenya, Mozambique, Rwanda, South Africa, Sudan, Tanzania, Yemen and Zimbabwe (Park et al., 2011; Singh et al., 2011). The "Ug99" lineage comprises at least seven pathotypes that differ for virulence on resistance genes *Sr21*, *Sr24*, *Sr31* and *Sr36* (Jin et al., 2009). Studies using DNA-based microsatellite markers showed that many of these pathotypes have identical fingerprints, consistent with them being recently derived from a common ancestor via single-step mutation (Visser et al., 2009; 2011). Significantly, surveys in Europe, Turkey, Pakistan and India over recent years have failed to detect any of these pathotypes.

The detailed knowledge of variability in *Pgt* in Australia that has come from pathogenicity surveys over the past 93 years has permitted a detailed understanding of *Sr* genes present in Australian wheat cultivars. This has allowed predictions concerning the responses of these cultivars to exotic threats such as "Ug99" that have been refined by field testing germplasm in Kenya with the assistance of the Kenyan Agricultural Research Institute from 2005-07. Because *Sr31* has not been used widely in Australia, the greatest impact of "Ug99" on germplasm to date has been due to virulence for *Sr30*, combined virulence for *Sr38* with other genes, and more recently, virulence for *Sr24* and *Sr36*. While virulences for *Sr30*, *Sr36* and *Sr38* have been detected in Australia, virulence for *Sr24* has not. The genes *Sr2*, *Sr12*, *Sr13*, *Sr22* and *Sr26*, effective against "Ug99" and derivatives, are important contributors to the resistance present in current Australian wheat germplasm.

Maintaining and improving current levels of rust control

It has been estimated that 50% of the cost of plant improvement involves breeding to maintain current yield and quality levels to meet the challenges of degrading growing environments and evolving pathotypes of major pathogens ("maintenance breeding"; McIntosh and Williamson 2004). Despite the low levels of stem rust over the past 35 years in Australia, *Pgt* remains a serious potential threat to wheat production. The development of virulence for *Sr38* in Western Australia in 2001 (Park, 2008) was a timely reminder in this regard. Despite the substantial successes of resistance breeding in controlling rust diseases in Australian wheat crops, the diseases continue to impact on production, with annual losses estimated at $147 million in 2009, most of which ($127 million) was attributed to stripe rust (Murray and Brennan, 2009).

Protecting the *ca.* $1 billion savings to the Australian wheat industry from resistance breeding and reducing the current impact of rust diseases will only be possible if resistance remains a priority in breeding programs, and if the wheat industry as a whole continues to support genetic approaches to rust control.

Achieving durable resistance to rust diseases

The concept of durable resistance was introduced about 40 years ago, following a breakdown in the slow rusting or APR of several English winter wheats to stripe rust, including Joss Cambier, and the continued effectiveness of resistance in several other cultivars including Cappelle Desprez and Hybrid de Bersee. The resistance in the latter was referred to as durable, and durable resistance defined as "resistance that remains effective when a cultivar is grown widely in environments favouring disease development" (Johnson 1978). Durable resistance is a descriptive term; it does not provide any explanation of the causes underlying long lasting resistance. It does, however, contain two conceptual elements, one being that there may be any of several underlying causes for durable resistance and the other that resistance that has remained effective for a long period of widespread use may not necessarily continue to do so in the future (Johnson, 1984).

Experience to date suggests that combinations of multiple effective resistance genes contribute to durability by lowering the chance of virulence matching gene combinations developing. Furthermore, some genes, notably the pleiotropic APR genes *Lr34/Yr18/Sr57/Pm38*, *Lr46/Yr29/Sr58/Pm39* and *Sr2/Yr30*, appear to be intrinsically durable. These pleiotropic APR genes have in many cases been used to great effect in conjunction with all stage resistance genes, providing backbone resistance that may also have contributed to increased durability of all stage resistance genes.

The advent of molecular genetics has provided new tools to assist in developing wheat cultivars with durable resistance. The number of resistance genes for which tightly linked high throughput DNA markers are available continues to increase, allowing marker assisted selection and greater efficiency in assembling gene combinations (Kuchel et al., 2007). The ability to incorporate multiple resistance genes by transformation will also become possible as more rust resistance genes are cloned. To date, genes/ alleles at seven rust resistance loci have been cloned from wheat: three conferring resistance to leaf rust (*Lr1*, Cloutier et al., 2007; *Lr10*, Feuillet et al., 2003; *Lr21*, Huang et al., 2003), one conferring resistance to stripe rust (*Yr36*, Fu et al., 2009), two conferring resistance to stem rust (*Sr33*, Periyannan et al., 2013; *Sr35*, Saintenac et al., 2013), and one conferring resistance to all three rusts and to powdery mildew (*Lr34/Yr18/Sr57*, Krattinger et al., 2009). An important consideration in this approach is, however, possible suppression of resistance genes. For example, recent studies showed that the powdery mildew susceptibility allele in wheat *Pm3C*, and three resistance alleles (*Pm3a*, *Pm3b* and *Pm3f*) all suppressed the resistance gene *Pm8* (Hurni et al., 2014). The mechanism of suppression was related to a post-translational mechanism; direct interaction between the two proteins produced by the resistance genes in tobacco suggested that the formation of a heteromeric protein complex may interfere with signal transmission in the defence reaction. While this example may be an exception, clearly any strategy based on combining cloned resistance genes will need to take this into consideration.

Improving rust diagnostics

In concluding his Presidential address to the Royal Society of NSW, Waterhouse (1938) said "Looking now to the future, from what has been set out it is apparent that specialisation is to be expected in each of the cereal rusts. And it must not be forgotten

that changes in the physiological races present may be looked for as time goes on. Any breeding programme designed to give control of rust should take fully into account this phenomenon of specialisation". These comments have been soundly verified by the work that has been done since on understanding rust pathogen variation, which remains a crucial part of genetic approaches in controlling these pathogens.

The methods developed to identify rust pathotypes have not changed greatly over the past 90 years or so. Using greenhouse assays, it takes about 2 to 3 weeks to identify a pathotype. In addition to providing new tools to expedite wheat breeding, molecular genetics is beginning to provide insights into rust pathogens at the genomic level (e.g. Duplessis et al., 2011). This has included the development of DNA-based molecular diagnostics. Although it is not yet possible to identify a rust pathotype using molecular diagnostics, whole genome sequencing has allowed the development of diagnostic microsatellite (Simple Sequence Repeat; SSR) markers that have allowed rapid placement of rust isolates into clonal lineage genotypes (e.g. Bailey et al., 2015). A preliminary study in which 70 *Pgt* isolates were sequenced has allowed the development of a *Pgt* SNP Chip (Illumina GoldenGate) containing 1,532 SNP, which can genotype an isolate of *Pgt* based on only a single pustule (Szabo et al., 2014). While such techniques are unlikely to replace greenhouse seedling assays, they will undoubtedly be used more routinely to provide rapid information on the identity of prevailing rust isolates as well as a greater understanding of rust pathogen population genetics and the development of new, sustainable approaches to rust control.

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