2. History and status of the wheat rusts

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Abstract

The rusts have been ongoing problems for wheat production probably since domestication of the crop about 8,000 years ago. Epidemics vary in size and frequency with host genotype and environment, wet years being 'rust' years. Although partial control in modern agriculture was achieved with resistant varieties, conditions favoring epidemics were made worse with the intensification of production and greater resistance gene uniformity in the host. The current Ug99 incident illustrates the situation of very widely adapted successful genotypes grown across huge areas in the presence of an ongoing threat from a recently emerged widely virulent and obviously highly aggressive pathotype of the stem rust pathogen. This paper addresses some of the history of cereal rusts and reviews underlying principles of host pathogen genetics, some of which are being neglected in the period of modern genetics.

Keywords
Cereal rusts • host : pathogen genetics • resistance • pathogenicity

Introduction

The cereal rusts can be serious diseases of the small grain winter cereals, including wheat, rye, triticale, oats and barley. The rusts of wheat attract the most attention because wheat is one of the two most important food crops for mankind. While all the cereal rust pathogens can be grouped as 'rust pathogens' because the different (Puccinia) species have many similarities, there are also clear differences in terms of life cycles, alternate hosts, host range and genetics.

The rusts and powdery mildews have been constant, irregular curses for farmers throughout the history of agriculture, but it was not until the 19th century that it was known that they were caused by fungi – the rust pathogens being Basidiomycetes. The discovery by de Bary that Puccinia graminis was a heteroecious species explained the much earlier observations that stem rust was often more serious when wheat was grown adjacent to hedgerows of barberry (Berberis vulgaris).

The next important step in understanding cereal rusts came in the late 19th Century when Eriksson and co-workers demonstrated the existence of formae specialis as variants of a single fungal species varying in ability to parasitize different host groups. For example, the wheat-attacking form of P. graminis, P. graminis f. sp. tritici (PGT), the rye-attacking form P. g. f. sp. secalis (PGS), and the oat-attacking form, P. g. f. sp. avenae (PGA). All of these forms are capable of completing their life cycles on the alternate (sexual stage) host, but differ widely in crossability. For example, PGT and PGS hybridize readily, but neither is crossable with PGA. By far the most attention has been given to these forms, and relationships with, and among, other forms are not so well known, but likely some are more closely related to PGA than to PGT or PGS.

During the 1910s, Stakman and co-workers in the USA showed that PGT was comprised of pathotypes (phenotypes, races, strains) with the ability to attack only certain combinations of Triticum genotypes, treating diploids, tetraploids and hexaploids as a group. Initially such pathotypes were given the status of genetically fixed entities more or less like species. Subsequently, pathotype variability was demonstrated in all cereal rust pathogen species and the number of pathotypes simply depended upon the number of host lines (later, differential genes or gene combinations), and the number of variations in infection type considered significant for any one host line, keeping in mind that the genetic bases of the various resistances were unknown. Thus, to some extent, the numbers of pathotypes depended on the amount of effort that investigators wished to invest in defining them. Nevertheless, pathotype identification and surveys became, and remain, routine activities in rust research laboratories worldwide.

Although de Bary described the various spore stages of P. graminis, P. coronata and P. triticina (PT) and identified their alternate hosts in Berberis spp., Rhamnus spp. and Thalictrum spp., respectively, it was Craigie in Canada who in 1927 demonstrated heterothallism and sexual reproduction in P. graminis, soon followed by Waterhouse (1929) in Australia with similar work with P. triticina.
In the 1940s and 1950s, host : pathogen genetics was put on a sound footing with the work on flax rust (an autoecious rust system) by Flor who, from genetic studies in both host and pathogen, showed that the expression of resistance in a host plant was specifically dependent upon the presence of a corresponding gene for avirulence in the pathogen. Any genetic or environmental (e.g. temperature) factor that prevented the presumed direct or indirect interaction of the gene products of the corresponding gene pairs resulted in a compatible disease response. I visualize a single corresponding gene pair (CGP) (Fig. 1) as the first law of host : pathogen genetics. The interactions shown in Fig. 1 assume homozygosity (or complete dominance) of both host resistance and pathogen avirulence alleles (cereal rust pathogens on cereals are dikaryotic, but behave as diploids). Differential phenotypic responses (infection types) can occur with incomplete dominance. For example, Samborski (1963) showed that a rare intermediate response produced by a *P. triticina* isolate on line Transfer with *Lr9* was due to heterozygosity of the corresponding *P9* locus in the pathogen.

If the above model is extended to a second CGP, the matrix rapidly becomes very complicated (Fig. 2) even when assuming homozygosity. However, this is the basis for the second law of host : pathogen genetics which is about the interaction of CGPs. When more than a single CGP is involved, the outcome is a phenotype that is as incompatible, or more incompatible, than the most incompatible of the individual participating CGPs. The use of these laws leads to the four basic experimental designs in host : pathogen genetics outlined by Browder (1971) (Table 1). One of the outcomes of Flor’s work was that the pathology community was very slow in appreciating its value; rather they saw it a phenomenon that had to be proved for each host : pathogen system before it could be applied experimentally. This was caused largely by a lack of awareness of genetics among pathologists. However, a reflective view of the gene-for-gene relationship is that it is the simplest logical explanation for genetic interactions between organisms, be they parasitic or otherwise. Such thinking led W.Q. Loegering (1985) to propose a sub-discipline of inter-organism genetics. Once the significance of the gene-for-gene interaction became known, there was an overuse of host genotype predictions based on multi-pathotype testing. People around the world could always postulate additional genes that did not fit the analysis, especially when constrained by the amount of variability among the pathotypes used. The necessary host genetic analyses required to validate the postulations were rarely performed.

### Table 1 Experimental designs used in host : pathogen genetics (after Browder 1971)

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### Centers of origin and global spread of wheat rust pathogens

There is general agreement that the centers of origin of pathogens are usually the same as the centers of origin of the host species (Karnal bunt may be an exception). In the case of heteroecious pathogens those areas should overlap with the alternate hosts. The pathogens (or at least components of their populations) then move from those areas along with the host species. To much of Europe, northern Africa and Asia, urediniospores could have been wind-borne from the Fertile Crescent. To more distal areas of southern Africa, the Americas and Australia, pathogens may have been wind-borne (at very low frequencies because...
the populations are quite different), but the original founding populations were more likely to have been transported in cereal hay (along with weed seed) used to feed animals. It seems highly unlikely that suitably adapted or adaptable pathogen populations would have been present on grasses (or alternate hosts) prior to colonization. Moreover, since the diseases usually appeared soon after colonization, the target areas of cultivated cereals would have been far too small to intercept what would have been extremely low levels of wind-borne viable spores.

The initial occurrences of stripe rust in South America (Chile) and North America were suggested to have arisen from native *P. striiformis* populations on indigenous grass communities. The South American pathotypes were very similar to European forms, but no exotic source was suggested for North American pathotypes.

In contrast to North America, Australian cereal rust researchers were always cognizant of their geographical isolation, and proposed the likelihood of introduced inoculum for a number of significant pathotype changes. For example, Watson and De Sousa (1983) presented both pathotype and meteorological evidence for the possibility of wind-borne spores from southern Africa, but origins of other instances of putative introductions of *PGT* and *PT* were never identified. While it has been generally assumed that there is no exchange of inoculum between northern and eastern Africa and southern Africa, a recent report (Visser et al. 2009) indicates that pathotypes closely related to Ug99 are not only present in South Africa, but are evolving in a parallel manner, and are current predominant pathotypes in that country.

The introductions of *P. striiformis* to eastern Australia in 1979 and 1998 (barley grass stripe rust), to Western Australia in 2002 and on to eastern Australia in 2003, South Africa in 1996, and to the USA in 2000 are likely examples of man-borne introductions. The 1979 introduction to Australia was probably from western Europe (based on pathotype identity), the barley grass rust and Western Australian introductions were likely from the USA (based on visitor frequencies), and the introduction to South Africa was probably from Turkey or neighboring areas (based on pathotype similarity). No origin has been proposed for the recent new group in the USA, but eastern Asia may be a possibility. The lack of commonality between differentials used in Europe, Australia and South Africa with those used in India, China and in the USA confounds the problem of pathotype comparisons, especially when we know that many of the genotypes used in each area carry combinations of (seedling) resistance genes rather than single genes. Whereas many will argue the need for molecular markers
to solve problems of identity, we should not lose sight of our very poor genetic understanding of variability in stripe rust resistance globally.

Australian researchers have proposed several recent introductions of PT, but in only one instance, was a probable geographical origin proposed. That one instance was pathotype 53-1,(6),(7),10,11, with virulence for Lr13, likely introduced from New Zealand; notably, it was a previously recent introduction to that country. Park (pers. comm.) identified pathotype 10-1,3,9,10,11,12 that was virulent to cv. Mackeller with Lr13. This pathotype was avirulent on seedlings of Morocco, a genotype that many of us know to be highly susceptible in the greenhouse and field, and perhaps look upon as a ‘universal suscept’. One might have expected it would be easy to trace the source of a PT population avirulent on seedlings of Morocco. Another example of this type is from personal experience in Japan. The island of Honshu cultivates only about 2,000 to 3,000 hectares of a single wheat cultivar, Norin 63. In 2000 McIntosh, Katto and Endo (unpublished) collected several samples of leaf rust from different locations on Honshu. All isolates were avirulent on seedlings of Thatcher, and a single gene for resistance was located on chromosome 2B using chromosome substitution lines. Such a pathotype had not been reported from other areas. Interestingly, very different pathotypes of PT were being reported from nearby Hokkaido where different varieties were grown and some breeding for leaf rust resistance had been undertaken. Obviously, in laboratories where Morocco and/or Thatcher are being used as susceptible hosts for initial increases of rust isolates, such pathotypes might not be detected even when present. There is probably no such thing as a ‘universal suscept’.

Somatic hybridization

Evidence has been presented for a possible role of somatic hybridization in the evolution of the cereal rust pathogens. The best evidence probably comes from the studies of Watson and others, initially at the University of Minnesota, and later in Australia using colored and rare pathotypes as sources of markers. Watson (1981) discussed the role of somatic hybridization in producing one group of PGT pathotypes in Australia. Following the introduction of PGS to Australia in about 1950, pathotypes that appeared to be somatic hybrids of PGS and PGT were isolated from grasses, especially Agropyron scabrum and barley. These isolates were very similar to sexual and somatic hybrids produced in the laboratory.

The distinction of putative hybrids derived from PGT and PGS was based on comparisons with the parents using key differential testers of both wheat and rye, and lots of experience.

Park et al. (1995) presented pathogenic, isozymic and RAPD data to propose that one pathotype of PT uniquely pathogenic to certain hybrid wheats, that carried Lr1 and were heterozygous for Lr13, was a somatic hybrid between two contemporary pathotypes.

Somatic hybrids were also reported in PST, but were not implicated in any significant role at the agricultural level.

Life cycles and disease cycles of the wheat rust pathogens

The stem rust and leaf rust pathogens are macrocyclic although the sexual stages probably no longer play significant roles in any major wheat-producing region. The alternate hosts of PG are Berberis and Mahonia spp. Historically, the telial/aecial cycles had important roles in that the telia are a resting (over-seasoning) stage, and were particularly important in areas with a long break between wheat crops, or with harsh environments that prevent over-seasoning of the uredinial stage. In the presence of barberry initial wheat-infecting aeciospores were obtained from barberry before incoming wind-borne urediniospores arrived from areas with milder climates. The second aspect is that sexual reproduction occurs on the alternate host, leading to new pathotypes; that is, generation of (homozygous) virulent genotypes from avirulent genotypes, and new combinations of virulence and avirulence alleles.

Leaf rust on wheat could be caused by more than one fungal species and there could be up to three different leaf rust diseases with each attacking three very different alternate host species, viz. Thalictrum spp. (Ranunculaceae), Anchusa spp. (Boraginaceae), Isopyrum fumaroides and Clematis spp. One form on tetraploid wheat in northern Africa and infecting Anchusa is considered to be P. recondita, usually recognized as the leaf rust pathogen of cereal rye, but the pathogenicity of wheat isolates on rye has not been reported. The rye leaf rust pathogen, P. recondita f. sp. recondita is highly avirulent on wheat, and P. triticina is likewise highly avirulent on rye, thus the relationships are quite different from the stem rust system. Relatively little seems to be known about the form infecting Isopyrum. Again, in contrast to stem rust, P. triticina does not attack barley and P. hordei, the barley leaf rust pathogen, does not attack wheat. An interesting question is whether genes in wheat conferring resistance to Anchusa-infecting pathotypes are effective in conferring resistance to P. triticina and vice versa. These relationships are important to discussions on host and non-host resistances.
The wheat stripe rust pathogen is microcyclic, having no known alternate host and only a uredinial cycle. Nevertheless, the species is equally variable in pathogenicity to other cereal rust pathogens. *Puccinia striiformis* f. sp. *tritici* (PST) was a relatively recent introduction to Victoria, Australia, in 1979, but there were two other notable incursions of *P. striiformis* with a form attacking barley grass (*Hordeum murinum* complex) occurring in eastern Australia in 1998, and a very different pathotype of PST appearing in Western Australia in 2002. It is of interest that although stripe rust had survived and spread in eastern Australia and New Zealand after 1979, it was not found in WA until 2002, largely paralleling what had been observed with the various cereal rust pathogens over many years of surveys by Waterhouse, Watson and co-workers.

Our interest in stripe rust on barley grass started from 1979 when we were interested in establishing if ancillary (uredinial) hosts might be important in the survival of PST. Although infected barley grass could be found in, and near, wheat fields it was never strongly implicated in over-season survival. However, Wellings (2007) isolated clones of PST that showed pathogenic differences on different isolates of barley grass. The PS identified in 1998 was clearly apparent by its moderate virulence on wheat differential Chinese 166 (Yr1) and its avirulence on the other PST differentials and most wheat lines, including Morocco. This form of PS subsequently became widely established on barley grass in eastern Australia and its frequencies of occurrence have waxed and waned with seasonal conditions. My particular interest was the origin of this pathogen, which can be considered a different *forma specialis*. Following earlier reports and discussions I formed the opinion that it came from the Americas. On a visit to Chile in 1999 I noted widespread stripe rusting of barley grass in the streets of Santiago and along roadsides to the south of the country. The infections were often long distances from wheat and barley suggesting that the pathogen was neither PST nor PSH. No laboratory work was possible. In 2003 I visited California with the specific purpose of finding barley grass rust. Widespread infections were found in non-cereal areas of western California and in the vineyard areas of the Napa valley where the understories included stripe rusted barley grass in situations where occasional wheat and barley plants were not rusted. Clearly the pathogen involved was not PST or PSH.

Many of the inheritance studies in wheat of wheat stripe rust resistance undertaken by Dr. XM Chen and colleagues at Washington State University, Pullman, include race PST-21, which is virulent on seedlings of Chinese 166 and no other differential. Such studies invariably detect new genes for resistance, some of which have been designated as regular wheat resistance genes. I contend that PST-21 is an isolate of ‘barley grass stripe rust’. It was originally collected from triticale in California in 1978, and despite subsequent collections in California on ‘wheat and/or triticale’ no actual sources were named. In the laboratory, it is avirulent on seedlings of Lemhi and all PNW PST differentials except Chinese 166. In genetic and molecular comparisons, PST-21 was always an extreme outlier, just as expected for a different *forma specialis*.

Whereas there is considerable knowledge about pathogen variability of the individual pathogen species on their cereal hosts, comparatively little is known about the specialization that might occur in the same species with respect to the alternate hosts, either at the intra- or interspecies levels, and likewise, relatively little is known about the genetics of interaction with ancillary hosts.

**Ancillary hosts**

Rust pathogen species vary in regard to the hosts they infect, a feature that cannot be ignored in relation to epidemiology and survival of inoculum. To be an effective contributor in the rust cycle of a particular host (or genotype), the pathogen must not only be capable of infection in a laboratory test, but also be capable of producing a significant and timely amount of urediniospores to be a significant source of inoculum to a cereal crop.

Barley is a host of PGT, but under Australian conditions it is seldom affected by stem rust in the absence of stem rust on wheat during the cropping season. Yet, out of season, stem rust can often be found on self-sown or regrowth barley. Thus, while barley is usually not affected by stem rust, it can be a significant carrier of inoculum through the summer for transfer to the wheat crop of the following season. During the cropping season, stem rust appears on barley crops later than on wheat, but as barley usually matures before wheat, the likelihood of losses is extremely low. Thus stem rust will be a problem in barley only when it is a problem in wheat or triticale. In North America, however, the situation appears to be at least partly different – pathotype QCC can sustain damaging epidemics on barley.

The presence of stem rust on off-season barley (and on *Agropyron scabrum*) in Australia always requires further investigation because the forms involved could be PGT, PGS or a more commonly encountered putative PGT x PGS somatic hybrid group, with only the first being a threat to wheat. Indeed, it seems that the hybrid forms are preferentially virulent on barley, leading Dr NH Luig to often refer to them as *PG hordei*. 

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Crop losses

The dynamics of rust epidemics parallel the dynamics of wildfires – the degree of fire damage is very much determined by fuel types and amounts (susceptible tissue), temperature and humidity (environment) and wind (weather patterns). In the case of an epidemic the timing and frequency of initial infection determine the initiation of the log phase of the epidemic, and influence the eventual crop loss. Like fire, epidemics feed on themselves in the sense that when the intensity is sufficiently great, even resistant materials (like green vegetation) will be affected to some degree. This effect was very clear in descriptions of the race 15B epidemics in North America in the 1950s, when various Hope (Sr2) and Thatcher derivatives were affected, only to be later used as some of our most important and sustainable sources of resistance.

Stem rust is usually considered to be the most damaging of the cereal rusts, but given extremely susceptible varieties covering large areas, early initial infection and favorable conditions, all three diseases can be extremely damaging with yield losses exceeding 70%. The history of significant stripe rust epidemics in China is quite instructive, because of the recurrent events based on overuse of single resistance sources. In the 1950s there was an epidemic on varieties with the Bima 1 (Yr1) source of resistance (estimated losses 6 mt), in 1964 the Mentana (Yr?) source was affected (losses 3.2 mt), in 1992 the 1BL.1RS (Yr9) source failed (losses 1.8 mt), and in 2002 in Sichuan it was the Fan 6 (Yr) source (losses 1.3 mt) (Wan et al. 2007). My prediction is that China is on the way to the next epidemic that will occur on wheats with Yr24/Yr26, an unfortunate circumstance because the two sources of resistance in the Nanjing-produced Haynaldia derivatives and CIMMYT synthetics were initially thought to represent genetic diversity. Such events are not unique as illustrated by the use of Lr24/Sr24 in Australia after 1980 when white seeded lines were first developed. By 2002 when virulence for Lr24 appeared, these genes were present in more than forty released and potential cultivars, despite warnings of genetic vulnerability. Why? Because those genes were highly effective, protected against two diseases, easy to use, and were being used by competing breeders.

Saari and Prescott (1985) summarized global losses to wheat rusts for the post-World War II period. Of particular interest is a map showing the migration of a PST pathotype virulent on ‘8156 cultivars’ (Kalyansona, Siete Cerros, PV18A, Indus 66, Laketch, Mivhor 77) from the Anatolian plateau to India during 1967 – 1970. To this day we do not know what gene was involved, but presumably it was Yr2.

Strategies to reduce or prevent losses include prevention of widespread over-season survival of inoculum by cultivation and grazing, targeting both the primary and ancillary host species. Forty years ago in Australia, we regularly collected stem and leaf rusted wheat plants on roadsides, railway lines and around public and farm sites protected from animals, but such findings are now quite rare in eastern Australia. The wheat plants surviving in such areas can be out-of-date genotypes. Resistant varieties are just as important in preventing local survival of inoculum as they are in preventing crop loss. Singh et al. (2007) described the potential benefit of Lr34 in preventing over-summer/early autumn survival of leaf rust. Clearly, chemicals can be used for crop protection, but are economically prohibitive until a potential crop yield is assured.

Control strategies must target the degree of susceptibility and the magnitude of use of susceptible genotypes through the availability of competitive resistant varieties. In many regions the removal of the more extremely susceptible genotypes (VS category) will have significant effects on both inoculum loads and crop loss. Any suggestion of legislative measures to control varieties is not acceptable.

Epidemiology of the rusts

Van der Plank (1963; 1968) and others illustrated the basic principles of epidemiology – widespread damaging epidemics occur as a result of large areas of susceptible hosts, high levels of initial inoculum and a continuing favorable environment. In some agricultural areas such conditions are difficult to avoid. For example, in Kenya wheat is continually planted and harvested, even in adjacent fields. As a consequence, inoculum passes from the maturing crop to the emerging crop. In China wheat can be continuously cropped at variable altitudes such that inoculum passes up and down the slopes in a single region. In both examples inoculum is available for wind-borne dispersal at any time of the year, and it is well known that urediniospores of Puccinia spp. can be transported over long distances. Wheat stripe rust very likely arrived in Australia about May or June 1979 (when it could have been present in wheat crops in Europe), when receptive crops in Victoria had emerged. Within two weeks of its initial discovery, we established by survey that it was already on a 600 Km front, and by the end of the 1979 crop season, it had been reported in central Queensland, in excess of 2,000 Km from its presumed initial focus of establishment. And this was a rust pathogen that was, at the time, considered to be relatively slow moving in Europe and North America.
It is useful to remind ourselves that stripe rust did not appear in the wheat belt of Western Australia until 2002, some 23 years after its occurrence in eastern Australia, but appeared in New Zealand only one year later. Interestingly, the pathotype in WA was a new exotic. It appeared in eastern Australia one year later. Many of the events reported for stripe rust were repeats of the patterns that Waterhouse and Watson and colleagues had established earlier with other cereal rust pathogens.

It is often considered that rust survival and spread follows certain \textit{Puccinia} pathways as has been described not only in Australia and New Zealand, but also in North America, China and India, and probably being repeated with Ug99 in Asia, with over-season survival either in milder latitudes or at higher altitudes in mountain areas. The over-season areas were considered as part of continuous cycles of inoculum or as inoculum exporting areas. Australia has no significant mountain areas where cereal rust pathogens would have an advantageous survival rate, and experience there suggests more or less random survival on regrowth and self-sown cereal hosts throughout the agricultural regions. If that is true in a dry country such as Australia, it is also more likely than usually acknowledged in many other areas. The long term survival of distinct pathotypic groups of \textit{P. triticina} in North America would also support inoculum survival in distinct regions extending at least to the Canadian border. This, of course, does not exclude wider exchanges of inoculum over longer time periods.

**Host : pathogen interaction**

In the early days of host : pathogen interaction studies people (mainly pathologists) observed that genotypes resistant in the field usually produced low responses in seedling tests performed with the same pathogen clones in a greenhouse. Thus the seedling test became an assay for resistance (or avirulence) under field and farm conditions. Moreover, they noted a range of highly repeatable and characteristic phenotypes varying from ‘immunity’ or no visible symptoms to large pustules that were characteristic of many genotypes that were susceptible in the field. These varying symptoms were described by Stakman and co-workers on a descriptive 0, ; (hypersensitive fleck), 1 to 4 scale that many of us continue to use at the present time, a scale that some people have now converted to 0 – 9 and increasingly used as a quantitative scale. Based on correlated observations between seedling responses at about 20ºC in the greenhouse and reactions under field conditions they decided that IT 3 and 4 represented compatibility and those below IT 3 represented incompatibility. These decisions were based completely on observation and involved no genetics. Unfortunately, this distinction is still used in many laboratories at the present time and many recorded IT 3 responses continue to be incorrectly interpreted, and examples will be discussed below.

The rusts are generally not serious diseases of seedlings and any research on seedlings is based on an assumption that the results will be highly correlated with responses in the field. In cases where seedlings at the first or second leaf stage are susceptible, but then become increasingly resistant as they develop we refer to such resistance as adult plant resistance or post-seedling resistance in contrast to seedling or whole of life resistance. These distinctions are not clear-cut. For example, \textit{Lr13} was originally (and unfortunately still is!!) described by some researchers as an APR, but we have no problem with scoring and interpreting it as a seedling resistance by using higher temperatures and interpreting certain IT 3 responses as low. On the other hand \textit{Lr18} was described as a seedling resistance. Under Australian conditions we learnt that this gene conferred a high seedling resistance only at low temperatures (<18ºC), and that it reversed its dominance over a range of temperatures, finally becoming ineffective at about 26ºC. All along the way, however, this gene was highly effective under the field conditions through which we worked. Thus in both examples we learnt how to conduct our greenhouse testing system (our laboratory assay systems) to maximize opportunities for identifying genes conferring resistance in our breeding nurseries.

Greenhouse studies on leaf rust and stem rust in North America were usually conducted as close to 20ºC as possible, obviously to control one parameter of the disease triangle. Indeed during my 1969-70 post-doctoral experience at the University of Missouri, I noted how rust work in the greenhouse largely ceased during the summer (too hot) and during the winter (too dark for dependence on natural light). The consequence was a relatively stable greenhouse environment. In Australia, we strived for a 12 month working cycle, and despite a less extreme ambient weather cycle, our greenhouse environments were in fact more variable. This allowed us to detect and interpret environmental effects that later became key to gene identification and manipulation.

After Flor placed host pathogen interactions on a genetic footing, and Sears provided the aneuploids stocks that permitted genes to be associated with chromosomes, we had the tools to permit a systematic cataloguing of resistance genes. In contrast to the various genes with which Flor worked where the high and low infection types were very distinctive, those produced in the cereal rust systems were often much more intermediate generating problems with decisions on effectiveness, but at the same time providing
distinctive phenotypes that often enabled or hastened gene identification – indeed this was a prime reason for producing the atlas of rust resistance genes in wheat (McIntosh et al. 1995).

Genetics is the study of inheritance of variation, and distinctions between high and low responses based on a century-old judgement that IT 3 was a cut-off between incompatibility and compatibility and is not genetically based. That cut-off should be based on contrasting phenotypes confirmed by progeny testing. An extreme example of this is the gene Sr23 which has no value per se in breeding but is an excellent ‘probe’ for the presence of Lr16. Against one PGT culture in the Sydney University collection, seedlings with this gene alone confer a necrotic low reaction (IT 1NN); with all other cultures, and the appropriate high light conditions the same host lines produce IT 3+N, very large (compatible?) pustules but with a characteristic and repeatable brown necrotic center, that is predictive of Sr23 and Lr16. Should we regard IT 3+N as high or low? Obviously, the decision is circumstantial and will vary with the purpose of the test.

Having thus used seedling response data as a probe for identifying variation, the relevance of that variation must be established using adult plants or a field plot situation. One recent study analysed ‘seedling resistance’ as a unique trait, and on the basis of separate QTL studies, concluded that the underlying genetic basis of variation was different from the genes controlling variation in response under field conditions. What is the practical use of a gene(s) that confers ‘seedling resistance’ to wheat breeding?

Pathogenicity studies

**Why conduct a pathogenicity survey?**

Reasons include: everybody else does it, we want a set of markers to track different clones in a complex asexually propagating population, we want to relate variation among clones to the likely responses of local cultivars, we want to isolate and identify clones for use in breeding nurseries, we want to identify and intercept clones that might be moving globally.

**Conducting pathogenicity surveys**

Having decided that a pathogenicity survey should be undertaken, decisions then have to be made as to what differentials should be used and that depends on which of the above questions are being asked. The number and type (isogenic lines vs cultivars) of differentials must be considered in the context of the area of the survey, keeping in mind that laboratory/greenhouse space (more samples or more differentials) and budgets will always be limiting. Should known genes for APR be used in pathogenicity surveys? It is interesting that all PT isolates virulent on Lr27 + Lr31 in seedling tests are virulent for Lr12 which is apparently identical to Lr31 but behaves as an independent APR gene. But we do not know how frequent Lr12 is in the host population and therefore whether using an Lr27 + Lr31 seedling tester is justifiable for that purpose.

The use of an international gene nomenclature system depends on the acceptance and (implicit) use of common differentials, but this is not acceptable to all researchers for a range of reasons, including personal, economic and historic ones. If PGT is taken as an example, genes Sr9a and Sr9d have never been effective in Australia, so it would be a wasted resource to include them in local routine surveys; similarly in North America Sr9g and perhaps Sr21, although it is used, might be considered of little use. On a global monitoring scale; however, such differentials are important because their various responses could be indicative of isolates from specific regions. Obviously, the detection of differences at this level is a current target for molecular markers capable of detecting clonal groups, but not of pathogenic variation within those groups.

Most modern race nomenclature systems are binary-based and place no value on differing low ITs for any particular differential or CGP. The original (see Stakman et al. 1962) system was based on actual infection types, but the variation on them was caused by the possibility of multiple resistance genes in some differentials as well as variation relating to single CGPs. This problem is another major reason why the Australian group has not adopted the North American nomenclature binary-based nomenclature system. Many of the PGT clonal groupings identified by Watson and co-workers were based on distinguishable differences in phenotypes produced by single CGPs including Sr6 and Sr15 (Watson and Luig 1968).

To overcome economic and space constraints, leaf rust surveys in Europe were based on inoculated leaf segments. For some differentials, mainly those giving very low seedling responses the method worked very well; for others giving intermediate and mesothetic responses on entire seedlings, there were problems and the genes involved, including Lr13 and Lr14a were not monitored despite being present (and possibly effective) at significant frequencies in European wheats.

**Can pathogenicity studies be field-based?**

Host genotype-based pathogenicity studies are possible and could be related to individual gene responses provided we included the single gene lines and various combination stocks to enable the identification of individual pathotypes; that is, the
gene combinations are necessary for the resolution of pathotypic mixtures. In an attempt to generate suitable gene combinations based on the Avocet S background I quickly came to realize that the use of such a set of lines on a large geographical scale will be confronted with problems of line purity and identification. Ideal surveys of this type also assume that only historically identified resistance genes are monitoring the variation, and that if those genes are seedling-effective factors, there are no additional genes for adult plant resistance. We already know that some of the Avocet S NILs (AvS+Yr1, AvS+Yr5, AvS+Yr10, AvS+Yr15, AvS+YrSp) carry Yr18. There are published reports on using the traditional stripe rust differentials in field nurseries, but as the majority of such differentials have APR genes additional to the seedling resistance factors, and the data obtained cannot easily be related to the current base of genetic knowledge or to the wheat genotypes being produced in the local area.

Virulence surveillance projects are special types of field-based surveys and are important sub-programs of the BGRI and DRRW. Here, care must be exercised to ensure that all participants are using correctly identified sets of tester genotypes if field data are to be collected across wide geographic areas. A problem with field-based surveys is that they are dependent on natural infection, many will not be infected, most will be sparsely infected, and therefore capable of providing inoculum for processing in laboratories, and only a few will generate sufficient host response differences for reliable recording. Whereas frequent lack of infection will be great for the local farmers, scientific institutions and scientists may soon be looking for cost-cutting and other ways to use their time in a more rewarding manner.

**Interpretations from pathogenicity survey data**

In recent years there has been an increasing tendency to interpret survey data simply from seedling infection type records without confirming what is being concluded. An example of this is interpretation of data for *Lr21*. A significant number of (particularly European) papers have reported virulence for *Lr21* based on IT 3 or 3-. In no case has a validation test of mature plants with this gene been carried out. I have discussed this issue with Dr J Kolmer on a number of occasions, such that he now emphatically states in his survey reports that *Lr21* continues to be effective. Given my contention that the seedling test is an assay, validation experiments must be undertaken, especially where there are no indicative field data to support such reports. Thus, as a challenge to the rust community I will state that *Lr21* is currently universally effective.

**Resistance**

**Concepts of resistance**

People from different backgrounds have different ways of conceiving resistance, and often the fact that what we see and interpret is based on host genotype, pathogen genotype and environment is ignored. Perhaps we should reflect on how we might think about resistance:

- as a pathologist: Stakman and co-workers made many decisions in the absence of genetics, yet we often cite their papers as a basis for our current phenotyping, especially in deciding what is high and what is low.
- as an epidemiologist: Here we are interested in delaying the increase and spread of rust at the field level or at the national level. Preventing over-season survival using *Lr34* could be important.
- as a geneticist: Genetics is about phenotypes and differences – IT 3 can be high or low depending on circumstances. A ‘super’ gene for a geneticist may not be so for a breeder.
- as a molecular geneticist: With extrapolations to genomics and function.
- as an economist: How much is a resistance gene or source worth?
- as a farmer: Availability of resistance at critical times, effectiveness of the resistance, cost of using it as part of risk analysis, knowing that rust is a ‘compound interest disease’ and that public risk/protection issues may be involved.
- as an agricultural scientist: An integration of the above.

**Types of resistance**

Resistances are divided into ‘seedling’, or ‘all stage resistance’, and APR, or post-seedling resistance, for convenience. Any resistance that cannot be characterized as a seedling resistance is designated APR; its time of onset depends on genotype and environment. Chen and co-workers at Washington State University describe a special type of APR that they designate HTAPR (high temperature APR), but my personal observations are that all APR to stripe rust is temperature-sensitive, and increases and decreases in sporulation on post-flowering plants occur with changing weather patterns.

Not all sources of APR are non-specific. *Lr12* is an excellent example of a genotype-specific (often called race-specific) APR to leaf rust. *Lr35*, an alien APR source transferred to wheat from *Aegilops speltoides*, might be predicted to be genotype-specific based on its hypersensitive response. Although not stated as such,
components of stripe rust APR, inadequately defined as Yr11, Yr12, Yr13 and Yr14, are almost certainly genotype-specific. Pathotypes allegedly virulent for these genes were isolated on the basis that they conferred increased rust levels, but the respective source hosts were not scored as highly susceptible to the new pathotypes, and genetic stocks with those genes individually were never produced or identified. Zadoks referred to ‘field races’ in a similar context. There are now emerging hints that certain QTLs for stripe rust resistance are genotype-specific (Rosewarne et al. 2008; Bansal and Bariana, pers comm).

Comparing the three rusts of wheat, stripe rust APRs are much more commonly encountered and reported, but increasing numbers of examples of leaf rust APRs are emerging in both hexaploid and tetraploid wheats. The reporting of effective APR to PGT pathotype Ug99 in the breeding program of RP Singh and others is most encouraging, but further genetic studies are urgently needed to determine if those resistances actually involve genes that are new.

**New sources of resistance and resistance to race Ug99**

Despite our increasing awareness of the characteristics of Ug99 and its potential as a significant threat to wheat, the discovery of new sources of resistance has been rather slow. Only one new gene for stem rust resistance (Lagudah et al. pers. comm.) has been documented in the last 10 years and the one documented before that (Sr45, Marais et al. 1998) is a duplicate of Sr21.

**Some examples and problems of stem rust resistance**

The gene Sr13 originally transferred to common wheat from *T. dicoccon* is easily recognized and widely effective in seedling tests (IT 2 to 3), but common wheat lines with this gene alone respond with relatively high MS responses in the field. Grain weight losses in rusted plots of lines with Sr13 can be as high as 50% relative to rust-protected controls indicating that this gene might have limited value. However, Australian cultivars Machete and Madden, which combine this gene with Sr2, are highly stem rust resistant. Although it has been used in stem rust resistance breeding for almost a century, Sr2 in some genetic backgrounds again confers only limited protection in rust nursery situations. However, its continued use and apparent durability over such a long period dictates that breeders should continue to use it in the future despite its limited field protection under experimental conditions, its sometimes excessive association with pseudo-black chaff symptoms, and its possible close repulsion linkage with Fhb1, an important gene for resistance to Fusarium head blight. Sr13 might be usefully utilized as part of a resistance package including Sr2, Sr13, or alleles at the same locus, is a very common gene for resistance in tetraploid wheats. Any attempt to isolate and characterize resistance genes in tetraploid wheat should focus on genes that are not Sr13, Sr8b or identified Sr9 alleles.

A significant problem with resistance genes that are moved from lower levels of ploidy to hexaploid wheat is a loss in level of effectiveness with increasing ploidy. For example, the infection types expressed in hexaploid lines with the *T. monococcum-*derived genes Sr21 and Sr22 are significantly higher than in the diploid sources. This was particularly true of Sr21 and is probably the reason that Ug99 was scored virulent for this gene. Apparently the decision to assess Ug99 as virulent for Sr21, for which the differential was a Sydney University line, was somewhat arbitrary, but the consequences can be significant for genetic research. According to the North American pathotype designation system, Ug99 was described as race TTKSK, the first ‘T’ indicating virulence for Sr21, or in host-talk, Sr21 is not effective. Two factors alerted me that there was a problem. Firstly, it was stated in the DRRW document that Sr21 was ineffective, whereas Sr45 was effective. Work in our laboratory had earlier shown that PGT pathotypes had the same specificities for these two genes indicating the genes were the same, although they derived from different species and were located in different homoeologous groups (chromosomes 2A and 1D, respectively). Secondy, hexaploid lines with Sr21 were resistant in the Ug99 nursery in Kenya in 2008. I have not been able to get actual data for the response of lines with Sr45 to Ug99 from the DRRW research document or elsewhere. Rouse and Jin (2008) reported a summary of a survey of accessions of *T. monococcum* tested with Ug99. Based on work conducted by The in Australia (The 1973, 1976), it was clear that the only gene that could give the frequency of resistance reported by Rouse and Jin was Sr21. Rouse and Jin also reported two other genes, both of which were previously identified, reported, and transferred to hexaploid wheat by us (McIntosh et al. 1984). Subsequent discussions with Tom Fetch (AFFRC, Canada) indicated that different isolates of Ug99 may vary in pathogenicity on seedlings of lines with Sr21 in which case I also predict they would correspondingly vary on seedlings with Sr45.

To some observers the above discussion may appear trivial. However, if we are to use the principles of host-pathogen genetics in a genetically meaningful and predictive way our phenotyping must be genetically based and correct. I am sure the resources used to test 1,062 accessions of einkorn wheat could have been used more effectively. Furthermore, the likely reason for the original mis-classification of Ug99 was based on an empirical interpretation of IT 3 as high.
Near-isogenic and single gene reference stocks

Near-isogenic lines are important resources for studies involving all traits, and partial sets of NILs are available for all three rust systems, viz., the Marquis and LMPG sets (Knott), Chinese Spring (Loeginger) and W2691 and Line E sets (Watson and Luig) for stem rust, the Thatcher series (Dyck) for leaf rust and the Avocet S (Wells) and Chinese sets for stripe rust. Unfortunately none of the sets are being extended for newly identified genes because production and conservation of such lines in the public domain is not seen as high profile science. Yet these are the very genetic resources that are required by basic researchers.

I therefore make a plea for international collaboration in the continuing and future development and conservation of appropriate NIL sets for all three wheat rusts. Perhaps the BGRI would be an ideal vehicle to promote and foster the development of such materials. In my role as the co-ordinator of the wheat gene catalogue I raised the issue of public availability of genetic stocks as a condition of naming genes. Although this was agreed in principle, my recent experience is that the collection of seed and getting it available in approved collections through barriers of import permits, export permits, and phytosanitary regulations at both ends is no longer an easy or cheap exercise.

One of the greatest hurdles to agreement on NIL development is agreement on genetic backgrounds – winter or spring wheat? Popular variety? Chinese Spring? Obviously that decision is based on the intended use of such lines. In encouraging the development of the Avocet S NILs for stripe rust work, I saw not only a very susceptible line at all growth stages, but also a widely adapted, easily handled pot plant.

Obviously, if markers are available, MAS can be used to generate the NILs – a good test of validation for both major genes and worthwhile QTLs – and a rust laboratory would not be required to complete the exercise.

Do we need to change the scientific method?

The classic and conservative approach to host: pathogen genetics and gene postulation was that when multi-pathotype test data arrays were identical we assumed that the resistance genes involved were the same until proven different. Given that there is a large volume of data from various laboratories on gene postulation, the likelihood of a rapid discovery of many new genes for stem rust resistance is relatively low. In the DRRW document the statements ‘Markers are also essential for determining genetic relationships of different varieties and sources of resistance. Two closely related varieties that possess unknown resistance alleles could have unknowingly derived their resistance from the same source’ are made to justify large-scale haplotyping of wheat genetic resources. Markers are not essential, although very helpful, and to me, the second sentence implies that we must prove genes to be the same, rather than to show they are different – a rather dangerous and hardly justifiable approach to the present problem.

Can we analyse by QTL and forget the pathogen population?

There is no doubt there are many situations where QTL analyses of disease data are justified. However, in situations where the analyses indicate one or two major QTLs accounting for most, say, 45% or greater, of the phenotypic variation across environments it might be worth considering a qualitative analysis and the likelihood that individual genes can be characterized. In two studies in Europe, one in common wheat and the other in durum, major QTLs were co-incident with the known position of Lr14a in chromosome 7B. As mentioned earlier pathogenicity for Lr14a cannot be determined using the leaf segment testing regime, but is known to occur from whole seedling tests. Swiss workers reported a QTL for leaf rust resistance in cultivar Forno that was co-incident with the position of Lr14a in chromosome 7BL. Cultivar Forno carries Lr14a (Pathan and Park 2006) thus suggesting the gene is Lr14a. A recent publication by Maccaserri et al. (2008) identified a major QTL (R² = 0.73) in the Lr14a region of durum cultivar Creso considered to have durable leaf rust resistance. Although specificity among pathogen isolates was demonstrated, and the seedling resistance was correlated with field response, the authors dismiss the likelihood that resistance was based on Lr14a because a virulent isolate was one of the 16 pooled to create the field epidemic. Unfortunately there was no sampling from the field to ensure the presence of that variant. The possibility of the gene being Lr14a was apparently considered not important, even though workers in Mexico had recently reported the presence of the gene in some of their durum populations – perhaps not completely surprising since it was originally transferred to hexaploid wheat from cultivated emmer. A reputation of durable resistance and a QTL analysis can easily lead to complacent attitudes.

Another study (Nay et al. 2008) involved a QTL analysis of seedling and adult plant (field) resistance in a backcross-derived population of a resistant synthetic/susceptible wheat cross using a single isolate of the pathogen in the greenhouse, and natural infection over several sites and seasons in the field. One wonders about the biological meaning of a mean seedling IT score and its standard deviation. A total of 11 QTL was identified, six at the seedling stage and seven for APR. One QTL
associated with Xbarc149-1D and having the largest effect was indicative of Lr21 which would likely come from Aegilops tauschii. However, it was suggested that the gene would have to be a new allele of Lr21 because the Tc+Lr21 NIL gave IT 2 (Thatcher IT 3) which was interpreted as a high reaction. As stated earlier, there is no evidence for virulence for Lr21 anywhere in the world. A suggestion for another QTL, on chromosome 1B, was Lr26. Whereas the single test culture used in the greenhouse was clearly avirulent for Lr26, presumably there would have been pathogenic variation or total virulence across the many field sites because of the wide use of 1BL.1RS cultivars in Europe over many years. In any case, a synthetic wheat involving T. dicoccoides as parent could not carry Lr26. Other naive comparisons are made. It seems that some research laboratories have become addicted to the power of the QTL approach, and the widespread assumption that QTLs represent non-specificity. The biological methodologies and ways of interpreting host : pathogen data, as well as the knowledge accumulated over the last 90 years seems to be forgotten.

**Alien segments in wheat usually retain their integrity**

A considerable number of wheat lines carry alien segments that have either contributed, or have potential of contributing, to agriculture. Those segments should generally not recombine with wheat chromosomes and genetic mapping should locate the translocation breakpoints. Yet I see published (and even more unpublished) genetic maps that seem to ignore cytogenetic realities. A recent paper by Mebrate et al. (2008) can be taken as an example because it provides the F$_2$ phenotypic data used to allegedly map Agropyron intermedium-derived gene Lr38 in chromosome 6DL. Apart from the fact that a result of 16:0 was treated as genotype RR and 15:1 was assumed to be Rr, the data for segregating lines are highly heterogeneous and should not have been treated as a uniform group of samples. If markers Xwmc773, Xcfd5 and Xcfd60 were dominant instead of co-dominant because the alien segment lacks amplifiable alleles, they should have co-segregated with Lr38. The authors proposed ‘a massive screen for polymorphic markers’ in close proximity to Lr38, but maybe a microscope and some cytology will be more biologically rewarding. The basic principles of Mendelian genetics and statistics should not be over-ridden with MAPMAKER.

**Non-host resistance**

The rally-call to the BGRI was the question of why rice has no rust disease raised by Dr Borlaug. I think Dr Borlaug can recall his days at the University of Minnesota when people were asking why cereal rye and oats were resistant to PGT and why wheat was resistant to PGS and PGA. It seems to me we can address the question of non-host resistance in two ways – try to understand the closer relationships just exampled, or search for effectors and receptors that might work at more distant levels.

Barley is a non-host (or maybe a near non-host in the terms used by Niks and co-workers) of wheat leaf rust. When we add the individual chromosomes of barley to wheat, no single addition line confers leaf rust resistance. Cereal rye is a non-host of wheat leaf rust; if we add rye chromosomes to wheat, some addition lines do have resistance (e.g. Petkus 1R lines with Lr26). By extension we might speculate the consequences of adding single chromosomes of oats, maize or rice to wheat and clearly we cannot predict what the consequences might be. Thus even if we can characterize the components of non-host resistance there is no assurance that they will function if placed in a wheat background. A search will then be needed to find the molecular tools that enable the expression of potential R genes in the recipient background. Various researchers have documented instances where rust and mildew resistances obviously expressed in potential donor species are not expressed in the amphiploids derived from them. An understanding of what is required for the expression of those resistances in wheat backgrounds would seem to be also relevant to understanding and perhaps utilizing some components of non-host resistance.

Working at the *formae speciales* level Australian rust workers showed that wheat carries resistance genes that are effective against PGS. The gene Sr18 in wheat is not only widely effective against PGS, but is present in most common wheat genotypes. Thus PGT must have virulence for this gene. The presence of this gene and a low number of others is adequate to largely protect wheat against PGS. At the same time it can be shown that rye carries a set of genes that protect it against PGT. All PGS and PGT x PGS isolates in Australia are virulent for Sr11 and polymorphic in pathogenicity for Sr5. Likewise, PGT clones vary in pathogenicity on certain cereal rye genotypes. The wheat lines W2691 and Line E were developed as genetic platforms allowing studies using isolates of both PGT and PGS and their hybrids.

Returning to the rice example one approach might follow the wheat : rye example by screening a wide representation of rice genetic resources, including Chinese wild rice, searching for unrelated accessions allowing some symptoms of infection. These could be intercrossed to permit a search for transgressive segregation, not forgetting the disease triangle, and thus the use of a geographic array of pathogen cultures and manipulation of the environment to enhance differences.
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