Probability of Mutation to Multiple Virulence and Durability of Resistance Gene Pyramids

C. C. Mundt

Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331-2902.
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A strategy for the management of race-specific resistance to plant disease is to combine (pyramid) two or more resistance genes into a single plant genotype. One view of this strategy is that resistance gene combinations increase the longevity of resistance due to a low probability of mutation to multiple virulence in the pathogen population. This probabilities hypothesis holds that mutations to virulence at different loci are independent and that the probability of mutation to multiple virulence is equal to the product of the rates of mutation to virulence for each avirulence/virulence locus. Using the probabilities hypothesis as a base, Wheeler and Diachun (24) postulated that a variety with four or five genes for resistance might provide stable resistance for centuries. Similarly, Schafer and Roelfs (18) calculated that four times the entire U. S. wheat (*Triticum aestivum* L.) hectareage would be required for a single mutant of *Puccinia graminis* Pers. var. *tritici* Eriks. & Henn. to arise that is virulent on a wheat cultivar with six resistance genes.

The probabilities hypothesis has been cited in the literature and is often taught in plant pathology courses. My own experience with teaching host plant resistance, however, indicates that an elaboration of the opposite view is needed. Hence, the purposes of this letter are to demonstrate that empirical data are not supportive of the probabilities hypothesis and to cite evidence countering the assumption of the probablities hypothesis that mutations to virulence at different loci are necessarily independent. In doing so, rust diseases will be used as examples because of the vast literature available on them and because resistance gene pyramids have been associated with an increase in the durability of race-specific resistance to wheat stem rust (8,18,23).

Empirical data. Green and Campbell (8) presented data on stem rust resistance gene identity, resistance gene number, and the durability of those resistance genes for wheat cultivars released during different time periods in Canada (Table 1). These data have been used to support the view that an increase in the number of resistance genes in modern (post-1951) wheat cultivars has contributed to the durability of stem rust resistance (8,18), although this view has not been universally accepted (10). The data of Green and Campbell (8) have been supplemented with more recent data (16) on the occurrence of the resistance gene *Sr*2 (Table 1). There at least three conditions that must be met if the data of Table 1 are to be considered supportive of the probabilities hypothesis. These are listed and evaluated below.

1. There should be a clear, positive association between the number of resistance genes in cultivars and the durability of those cultivars. Table 1 shows no clear association between resistance gene number and durability of resistance to stem rust. For example, three of the four cultivars that possess four or more resistance genes and which were licensed between 1951 and 1978 have maintained their resistance. The fourth cultivar, however, has not. Of the two modern cultivars that have not maintained their resistance (Canthatch and Pitic 62), one possesses five identified resistance genes and the other possesses two. Further, five cultivars licensed before 1951 possess four identified resistance genes. Three of these proved to be nondurable, while the other two were already susceptible when released for commercial use.

2. Previous use of resistance genes combined in durable cultivars should not have resulted in previous selection for corresponding virulence in the pathogen population. If such selection occurs, then the durability of a cultivar may be due to factors other than a low probability of combining corresponding virulence in the pathogen. Table 1 shows that there was much opportunity for selection of virulence before resistance gene combinations were deployed during the 1951–1978 period. Eight of the 13 identified resistance genes in the post-1951 cultivars of Table 1 are listed as being deployed in licensed cultivars before that time. A striking example is the cultivar Selkirk, which occupied as much as 85% of the wheat area of Manitoba, Canada, during the period 1955–1965 (8). Four of the six resistance genes listed for Selkirk in Table 1 were used previously in the cultivars Renown and Redman, which were licensed in 1937 and 1946, respectively (Table 1). Because virulence to Renown and Redman had already been detected when Selkirk was licensed in 1953 (8), Selkirk had only two new genes for resistance based on the data of Table 1, rather than six.

3. There should be an absence of factors other than gene number that could account for the durability of resistance. Table 1 shows that the presence of the gene *Sr*6 is more closely associated with durability of resistance than is the number of resistance genes a cultivar possesses. For example, of the seven modern (post-1951) wheat cultivars that are listed in Table 1, five had maintained their resistance up to 1978; of these five cultivars, four possess the *Sr*6 gene. Of the two modern cultivars that failed to maintain effective resistance, one possesses five resistance genes and the other possesses two; neither possesses the *Sr*6 gene. None of the five four-gene cultivars licensed before 1951 possess the *Sr*6 gene.

Virulence against *Sr*6 has commonly been found in populations of *P. g. tritici* in North America (7). Thus, *Sr*6 alone cannot account for the durability of cultivars listed in Table 1. However, it has been noted that certain virulence combinations in plant pathogen populations may be associated with reduced fitness of a pathogen (8,22,26,27), and it has previously been suggested that such a reduction in fitness is associated with the durability of wheat cultivars possessing the *Sr*6 gene in combination with other resistance genes (8,22). In Australia, cultivars possessing *Sr*6 in combination *SrTt*1 have been durable (23). It is particularly relevant to note that races virulent against all six resistance genes combined in the cultivar Selkirk have been detected in North America, but were not sufficiently fit to be maintained at high frequency (8). Thus, the durability of cultivars such as Selkirk may be caused by reduced fitness of corresponding rust races, rather than a low probability of that race occurring. Day (4, p. 180) very concisely summarized the difficulty of distinguishing between mutation and fitness effects by stating, "Practical experience does not usually enable one to discriminate between low mutation rate and low fitness of a majority of virulent mutants since the end points are likely to be the same: the virulent mutant fails to become established."

Johnson (10) previously considered the data of Green and Campbell (8) and also concluded that gene number was not highly associated with durability of stem rust resistance. He suggested

that the durability of Selkirk may be due to the presence of unidentified resistance genes such as *Sr*2, an adult plant resistance gene that was apparently not identified in Selkirk until after the publication of Green and Campbell's (8) data. However, a more recent catalog of stem rust resistance genes (16) shows that the cultivars Renown and Redman, which were susceptible when Selkirk was released, also possess *Sr*2. Therefore, *Sr*2 alone cannot explain the durability of Selkirk. One cannot, of course, rule out the possibility of other, unidentified resistance genes playing a role in the durability of resistance to stem rust.

Assumption of independence of mutations to virulence. A critical assumption of the probabilities hypothesis is that mutations from avirulence to virulence occur independently. The small amount of empirical evidence presently available, however, shows that mutations to virulence are not always independent in rust fungi. For example, Statler (21) studied mutations in an isolate of *P. recondita* treated with the mutagen *N*-methyl-*N'*-Nitro-*N*-nitrosoguanisine. A total of 15 cultures with mutations from avirulence to virulence were isolated. The number of cultures with one, two, three, four, five, and six mutations from avirulence to virulence at different loci was two, one, three, four, four, and one, respectively. It would be highly unlikely to obtain such a large number of isolates with multiple virulence changes if mutation at each locus were independent. Similarly, Griffiths and Carr (9) irradiated a population of *Puccinia coronata* f. sp. *avenae* uredospores with ultraviolet light and inoculated the irradiated spores on 10 differential oat (*Avena sativa*) cultivars. The number of infections produced on the susceptible control indicates that only about 150 viable uredospores were screened, yet the one mutant isolate obtained was virulent on six differential cultivars to which the original culture was avirulent, and avirulent on a seventh to which the original was virulent. These differential cultivars were reported to each possess a different factor for resistance to *P. c. avenae* (9). Because these were not single-gene differentials, however, the number of virulence mutations could have been either less or more than seven. Flor (6) studied the virulence of 198 progeny resulting from selfing two isolates of *Melampsora lini* that were induced to mutate to virulence on the flax (*Linum usitatissimum*) cultivar Koto by X-ray treatment. Sixteen of these progeny were also virulent on the cultivars Abyssinian and Leona, even though the original isolates that were irradiated were homozygous avirulent on these two host genotypes. In addition, all 16 of these isolates were virulent on the cultivar Akmolinsk, to which the original isolates were heterozgous for avirulence.

Flor (6) explained his results with X-ray induced mutants of *M. lini* as a deletion that affected four closely linked genes for avirulence. Given the evidence that the specificity in host-parasite interactions is conditioned by genes for avirulence and that virulence is the result of a lack of function (3,5,6), a single deletion mutation that affected more than one avirulence/virulence locus could result in simultaneous, dependent mutations from avirulence to virulence. In rust fungi, it has generally been believed "that different genes for virulence are usually unlinked and are not allelic" (4, p. 67). However, Burnett (2, p. 210) noted that "there is insufficient data on the position and linkage relationships of virulence genes in any pathogenic fungus for this hypothesis to be examined adequately." Given the large number of avirulence genes in the rusts, and accepting the cytological evidence for six chromosomes in *P. tritici* (14), there may be significant linkage among avirulence genes in this organism. In fact, examples of virulence linkages and multiple allelism in rust fungi are beginning to accumulate (12,17,19,20).

Mutation by deletion is an attractive hypothesis to explain the results of the mutation studies described above because such a mechanism is simple and is compatible with current concepts of the genetics of host-parasite interactions. Such mutations would tend to be more detrimental to the pathogen than would point mutations at single virulence loci, thus reducing the probability of combining multiple virulence with high fitness. Of course, only genes on the same chromosome would be expected to be influenced by the same deletion, but little is known of the chromosomal location of virulence genes.

Other mechanisms of attaining simultaneous changes to virulence at different loci may also exist. For example, Jones (11) recently reported on a dominant gene in *M. lini* that inhibits the expression of five different avirulence genes. Thus, a single mutation from noninhibition to inhibition could result in the simultaneous inactivation of five different avirulence alleles. Molecular approaches may further identify mechanisms for the nonindependence of virulence alleles. For example, Wieczorek et al (25) have shown the rat α-tropomyosin gene to possess a complex structure of at least 13 exons and two different 3′ ends. This structure enables the gene to code for six to eight different messenger RNAs (mRNAs) that produce different isoforms of tropomyosin, a protein involved with the contractile apparatus of cells. Alternative splicing of mRNA results in tissue-specific expression, such that different isoforms of α-tropomyosin are produced in nonmuscle, smooth muscle, and striated muscle cells. Previously, it was assumed that a different set of genes coded for tropomyosin production in each of these tissues. A similar process might occur in plant host-parasite systems whereby a single gene for avirulence may code for different avirulence products, depending on the genotype of the host that it interacts with.

Certainly, there are mechanisms of rapid change to multiple virulence in plant pathogen populations. A more important and difficult question, however, is whether such changes are of importance in nature, or merely rare events with little practical relevance. The importance and difficulty of this question can be demonstrated by the population dynamics of *P. g. tritici* in Australia. Some Australian rust workers have considered small changes to virulence in *P. g. tritici* to be due to mutation of existing races, and large virulence changes to be due to immigrants from another continent (1,23). For example, Burdon et al (1) provided evidence concerning the origins of stem rust races in Australia through the use of isozyme analyses. They found that new races appearing from 1969 onwards had isozyme patterns (based on eight enzymes) identical to standard race 21, which had been known in Australia since 1954, and also to races on the African continent. The post-1969 races were virulent at several more loci than race 21, but had a virulence pattern similar to races extant in Africa. They therefore concluded that the post-1969 races originated as immigrants from Africa.

An alternative hypothesis is that post-1969 races of *P. g tritici*

TABLE 1. Characteristics of spring wheat cultivars with known stem rust resistance genes and released for production in western Canada during three time periods[a]

Cultivar	Year licensed	Rust reaction[b] When licensed	Rust reaction[b] 1978	Known resistance genes
Licensed prior to 1935				
Kota	Pre-1923	S	S	*Sr*28
Marquis	Pre-1923	S	S	*Sr*7b, 18, 19, 20
Red Bobs	1926	S	S	*Sr*7b, 10
Reliance	1932	S	S	*Sr*5, 16, 18, 20
Licensed 1935–1950				
Thatcher	1935	R	S	*Sr*5, 9g, 12, 16
Renown	1937	R	S	*Sr*2, 7b, 9d, 17
Redman	1946	R	S	*Sr*2, 7b, 9d, 17
Lee	1950	R	S	*Sr*9g, 11
Licensed 1951–1978				
Selkirk	1953	R	R	*Sr*2, 6, 7b, 9d, 17, 23
Canthatch	1959	MR	S	*Sr*5, 7a, 9g, 12, 16
Pembina	1959	R	R	*Sr*2, 5, 6, plus
Manitou	1965	R	R	*Sr*5, 6, 7a, plus
Neepawa	1969	R	R	*Sr*5, plus
Pitic 62	1969	R	S	*Sr*8, 9b
Napayo	1972	R	R	*Sr*5, 6, 7a, plus

[a] Data are from Green and Campbell (8) with the exception of those concerning *Sr*2, which were derived from Roelfs (16).
[b] S = susceptible, R = resistant, and MR = moderately resistant.

in Austalia arose from a large change in virulence in the indigenous population of standard race 21 through one of the mechanisms described above. The similarity of virulence pattern for the post-1969 Australian races and the African races may simply represent a commonly occurring virulence change in *P. g. tritici*, since certain mutations to virulence in cereal rust populations occur much more commonly than others (21,23). This view is supported by the fact that the *P. c. avenae* mutant derived by Griffiths and Carr (9) through ultraviolet irradiation, and which expressed virulence changes on seven cultivars, had a virulence spectrum identical to races isolated from the field in Argentina and Israel.

Conclusions. Data evaluated in this letter cast doubt on the probabilities hypothesis as the primary mechanism for the durability of resistance gene pyramids. The influence of specific virulence combinations on pathogen fitness and the effect of mutation to multiple virulence on fitness (15) may be at least as important as the probability that a change to multiple virulence will occur. Perhaps the most important point to be derived from this letter, however, is that plant pathologists lack adequate information to properly evaluate the probabilities hypothesis. Empirical data such as those shown in Table 1 are inconclusive, at best. In fact, it would very difficult to develop the biological material necessary to test the hypothesis empirically. Similarly, our current understanding of the population genetics of plant pathogens precludes an adequate evaluation of the probabilities hypothesis from a theoretical perspective.

This letter is not offered as a "correct" or complete evaluation of the probabilities hypothesis. There are certainly other assumptions of the hypothesis that could also be questioned (e.g., mutation is the only source of genetic variation for plant pathogens, virulence genes are at low frequency in the absence of corresponding resistance). Nevertheless, I hope that the letter will make some contribution to understanding the influence of resistance gene combinations on plant pathogen populations.

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