Letter to the Editor

Probability of Mutation to Multiple Virulence and Durability of Resistance Gene Pyramids: Further Comments

C. C. Mundt

Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331-2902.
Paper 9457 of the journal series of the Oregon Agricultural Experiment Station.
Accepted for publication 10 December 1990 (submitted for electronic processing).

Kolmer et al (7) have questioned conclusions of my letter to the editor (14) concerning mechanisms contributing to the durability of resistance gene pyramids. The purposes of this letter are to clarify the intent of my original one and to demonstrate that the data discussed by Kolmer et al (7) do not alter the validity of my view.

Kolmer et al (7) emphasized a defense of resistance gene pyramiding as a breeding strategy to obtain more durable resistance. Clearly, there are breeding programs focusing on resistance gene combinations that have been very successful in increasing the durability of resistance (3,17–19), and the accomplishments of these programs are highly impressive. The purpose of my original letter (14), however, was not to comment on the value of resistance gene pyramiding as a breeding strategy. Rather, my intent was to discuss mechanisms by which resistance gene combinations may contribute to the durability of resistance. Potential mechanisms include 1) a low probability that a pathogen can simultaneously mutate to virulence at loci corresponding to the combined resistance genes, 2) fitness disadvantages caused by specific combinations of virulence in the pathogen, 3) the difficulty of combining multiple virulence mutations with high pathogen fitness, and 4) the chance discovery of single resistance genes or combinations of small numbers of resistance genes that are more durable than average. Breeding for resistance gene combinations will increase the durability of resistance, on average, if any one or any combination of these mechanisms is operative. The sole purpose of my original letter was to use rust diseases as an example to demonstrate that there is little evidence to support the first mechanism, which I called the probabilities hypothesis, as the primary mechanism responsible for the durability of resistance gene combinations. To avoid further confusion I will formally define the probabilities hypothesis: *Cultivars possessing multiple race-specific resistance genes owe their durability to a low probability of the pathogen's mutating to virulence independently at avirulence/virulence loci corresponding to those resistance genes; the probability of mutation to virulence at multiple loci is equal to the product of the mutation rates for each locus.*

Kolmer et al (7) noted that the spring wheat (*Triticum aestivum* L.) cultivars Thatcher and Renown have been moderately resistant to stem rust (caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn.) since 1963 and objected to my listing these cultivars as susceptible in 1978. In Table 1 of my letter (14), and in Table 2 of Green and Campbell (3), a susceptible rating for a cultivar indicates that its resistance had "broken down" by 1978 as a result of selection for a race virulent to that cultivar. A susceptible rating does not necessarily mean that the cultivar was heavily rusted specifically in the year 1978. Thatcher and Renown clearly demonstrated nondurable resistance in the 1950s and early 1960s (3). The "moderate resistance" noted by Kolmer et al (7) for these two cultivars in nurseries since 1963 was merely because inoculum virulent to them was not being produced in commercial fields. Green and Campbell (3) noted that the percentage of the Manitoba wheat hectarage planted with Thatcher was 61.8, 1.6, and 0% in 1941, 1961, and 1977, respectively. The corresponding values for Renown were 22.1%, trace, and 0%, respectively. Thus, there was little or no inoculum in the air spora to produce infections on Thatcher and Renown in nurseries in later years.

Kolmer et al (7) also questioned my contention (14) that there is a weak association between the durability of stem rust resistance and the number of resistance genes listed by Green and Campbell (3) for different wheat cultivars. I did not intend to suggest that resistance gene combinations play no role in the durability of resistance to stem rust in North America. My intended point was that if the durability of resistance can be explained primarily by the probabilities hypothesis, there should be a strong, quantitative relationship between gene number and durability. Such a relationship does not exist in the data of Green and Campbell (3). Further, the additional stem rust data discussed by Kolmer et al (7) do not counter my view. Kolmer et al (7) demonstrated that most stem rust resistance genes in North American spring wheat cultivars are ineffective against commonly occurring races of stem rust. They showed that resistance is often due to a single gene or a pair of genes. Thus, they demonstrated no clear relationship between the total number of resistance genes and the durability of resistance. Further, their discussion of resistance in Thatcher and Renown is irrelevant, as this resistance cannot be considered durable sensu Johnson (5). Johnson (5) defined durable resistance as "resistance that remains effective while a cultivar possessing it is widely cultivated." As I discussed earlier in this letter, resistance in Thatcher and Renown was nondurable when they were widely cultivated.

Kolmer et al (7) claimed that I associated the gene *Sr6* alone with the durability of resistance to stem rust in western Canada. They discussed evidence to show that "the presence or absence of *Sr6* alone has not necessarily determined the durability of stem rust resistance in western Canadian wheats." This criticism is peculiar, as I clearly stated (14) that "*Sr6* alone cannot account for the durability of cultivars listed in Table 1." My point was to demonstrate that there are factors other than gene number (in this case I used the example of the combination of *Sr6* with other resistance genes) that are more closely associated with the durability of resistance than is the number of resistance genes that a cultivar possesses.

The wheat leaf rust data presented by Kolmer et al (7) are clearly not supportive of the probabilities hypothesis as the primary mechanism for the durability of leaf rust resistance. They noted that the cultivars Chris and Era (*Lr13* + *Lr34*) and Columbus (*Lr13* + *Lr16*) have been highly resistant since 1966, 1972, and 1980, respectively. On the other hand, the cultivars Manitou and Neepawa (released in 1965 and 1969, respectively) possess only *Lr13* and showed susceptible reactions by 1974. Kolmer et al (7) claimed that these data support the probabilities hypothesis. However, cultivars possessing only *Lr13* occupied 2, 77, and 69% of the hard red spring wheat area of the Canadian prairies in 1966, 1975, and 1987, respectively (6). Thus, there must have been considerable inoculum of races with virulence to *Lr13* in the Canadian prairies, and only a single mutation would be required for the pathogen to be able to attack Chris, Era, or Columbus. Obviously, the durability of leaf rust resistance in Chris, Era, and Columbus is not related to the probability of multiple virulence mutations occurring in the pathogen.

There are other weaknesses in the leaf rust example used by Kolmer et al (7). First, they did not (and it was probably not possible to) provide an adequate analysis of the durability of

Lr16 and *Lr34* when deployed singly. Their Table 3 lists near-isogenic lines of Thatcher that possess either *Lr16* or *Lr34* alone. These lines have maintained resistance in nurseries since 1970 and 1974, respectively. Since the genes have apparently not been deployed singly in cultivars, however, they would have had no significant selective influence on the leaf rust population. A further weakness of the leaf rust example of Kolmer et al (7) is their failure to account for the nondurability of leaf rust resistance in the cultivar Selkirk, which possesses both *Lr10* and *Lr16* (17).

I agree with Kolmer et al (7) that caution should be advised when extrapolating results of laboratory mutagenesis studies to the field. However, their analysis of the rust mutagenesis data that I discussed (14) is not entirely correct. Kolmer et al (7) claimed that I only included data from studies using the "powerful mutagens" *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and X rays, and they failed to note that I also included a study using ultraviolet radiation as a mutagen. Also, their complaint that mutagenic agents induce higher than natural mutation rates is irrelevant. It is the relative rate of single to multiple virulence mutations that is important. In all of the studies I discussed (14), multiple mutations to virulence occurred at a very high frequency relative to mutations at single virulence loci, regardless of the mutagenic agent. One cannot be sure that the same relationship between single and multiple mutations holds for natural field populations, and this is a valid criticism of my original letter (14). Nevertheless, virulence changes induced at single loci by artificial mutagenesis have often been found to occur more frequently at the loci that mutate most frequently under natural conditions (1,8). The same could be true of more complex mutations. As noted in my original letter (14), it is significant that an isolate of *P. coronata* var. *avenae* exposed to ultraviolet radiation expressed virulence changes on seven differential cultivars and had a virulence pattern identical to that of races isolated from the field in Argentina and Israel (4).

Increasingly, information is becoming available to show that there are potential mechanisms of virulence variation other than point mutations in fungi. Row et al (16) noted that lagging chromosomes are common during mitotic divisions in *Pyricularia oryzae*. They postulated that lagging chromosomes could result in the production of aneuploids and, hence, virulence variation. Restriction fragment length polymorphism analysis detected deletion mutations in 9% of *Septoria tritici* isolates studied (11), a level much higher than that for higher organisms. Our knowledge of chromosomal variation in fungi has increased dramatically in recent months through the use of pulsed-field gel electrophoresis, which allows for physical separation of chromosome-sized DNA fragments. A recent review of these studies indicated a large degree of polymorphism in both the number and the size of chromosomes of plant-pathogenic fungi (13). Such polymorphism could play an important role in virulence variation. For example, McCluskey and Mills (10) found unique electrophoretic karyotypes for 14 races of *Ustilago hordei*, with the chromosome number varying from a minimum of 15 to a maximum of 19 and total DNA varying 1.35-fold among races. Significantly, karyotypes were almost always invariant among individuals from the same meiotic tetrad and among tetrads of the same race. In another system (*Nectria haematococca*, anamorph *Fusarium solani*) it was shown that host range can be changed by the loss of a small chromosome containing a gene for pisatin demethylation (12). Unfortunately, very little information is currently available on chromosomal polymorphisms for the rust fungi, which I used as primary examples in my original letter to the editor (14).

Finally, Kolmer et al (7) claimed, "there is little evidence that multiple changes in virulence commonly occur in field populations of cereal rust fungi." The data for wheat leaf rust (6) and oat stem rust (9) that they cited are not very relevant, as these are pathogens against which resistance genes have been added to cultivars mostly one at a time (6,9). Clearly, single virulence changes will be more likely to occur than more complex ones. Thus, when resistance genes are released one at a time, one would expect selection to be predominately for single virulence differences in the pathogen. It is of more interest to evaluate data for wheat stem rust, against which more complex resistances have been deployed (3,18). Kolmer et al (7) noted the work of Green (2), who showed convincing evidence that virulence changes in race group 15B-1L of *P. g. tritici* were due to a series of single virulence changes. What Kolmer et al (7) failed to mention, however, was that Green (2) evaluated data for two other race groups of *P. g. tritici* (11-32 and 17-29) and found large differences in the numbers of virulence genes among races in both of these groups, but concluded that the races showed "no chronological, evolutionary, stepwise pattern" and that it was "not possible to find an evolutionary series of races differing by single virulence."

Green (2) also noted two major events in the evolution of *P. g. tritici* that could have involved radical mutational changes to multiple virulence. The first was the appearance in 1950 of race C10 (15B-1), which attacked all resistant cultivars in Canada except one despite the fact that "many varieties had been selected in the field and had complex resistance" (2). The second event was the relationship of race C9 (15B-1L) to its putative ancestor, race C10 (15B-1). Initial analyses indicated that these two races differ by virulence on only two of the standard differential cultivars. However, the later availability of single-gene differentials showed that they differed by at least five different virulence genes. There are, of course, many possible mechanisms that could have caused these two events. Nonetheless, they suggest that important events in pathogen evolution may not always result from simple, single-gene changes.

I emphasize that I am not suggesting radical changes to multiple virulence to be the most common type of virulence change detected in virulence surveys. My emphasis is that mutants combining virulence against many resistance genes may occur, but there are mechanisms that prevent these genotypes from dominating. To quote from my original letter (14), "the influence of specific virulence combinations on pathogen fitness and the effect of mutation to multiple virulence on fitness may be at least as important as the probability that a change to multiple virulence will occur." There are certainly other mechanisms that are operative.

In conclusion, I agree with Kolmer et al (7) that "durable resistance to both stem rust and leaf rust of wheat has been achieved by combinations of resistance genes." The single purpose of my original letter (14), however, was to demonstrate that there is little evidence to support the hypothesis that such durability derives primarily from a low probability of a pathogen's mutating to virulence simultaneously at multiple virulence loci. My view is compatible with that of Person et al (15), who came to a similar conclusion for different reasons and stated, "we do not agree with those who have assumed that selection for the rare matching genotype would be made possible only after a highly improbable sequence of mutational events has occurred." The evidence discussed by Kolmer et al (7) is mostly irrelevant to or supportive of this view.

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