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# The population genetics of plant pathogens and breeding strategies for durable resistance

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**Summary**

The durability of disease resistance is affected by the evolutionary potential of the pathogen population. Pathogens with a high evolutionary potential are more likely to overcome genetic resistance than pathogens with a low evolutionary potential. We will propose a set of guidelines to predict the evolutionary potential of pathogen populations based on analysis of their genetic structure. Under our model of pathogen evolution, the two most important parameters to consider are reproduction/mating system and gene/genotype flow. Pathogens that pose the greatest risk of breaking down resistance genes are those that possess a mixed reproduction system, with at least one sexual cycle per growing season and asexual reproduction during the epidemic phase, and a high potential for gene flow. The lowest risk pathogens are those with strict asexual reproduction and low potential for gene flow. We will present examples of high- and low-risk pathogens. Knowledge of the population genetic structure of the pathogen may offer insight into the best breeding strategy for durable resistance. We will present broad guidelines suggesting a rational method for breeding durable resistance according to the population genetics of the pathogen.

*Abbreviations:* GFG – gene-for-gene; MGR – major gene resistance; QR – quantitative resistance

## Introduction

We will address two questions in this paper. 1. How does the genetic structure of pathogen populations affect the durability of genetic resistance? 2. How can knowledge of pathogen genetic structure be used to guide breeding programs to lead to durable resistance?

We hope to convince the reader that the principles of population genetics can be used to guide the development of control strategies for plant pathogens in agroecosystems. These principles can be applied to all pathogens, including fungi, bacteria, nematodes, and viruses. One of our aims in this paper is to illustrate how principles of population genetics can be applied to guide resistance-breeding strategies. Though the main focus will be on plant genetic resistance, these principles should be equally relevant to management of fungicides and antibiotics in agroecosystems, as well as to disease management through cultural methods and quarantines. A second goal in this paper is to develop a flexible framework in which to consider the population genetics of plant pathogens and then relate that framework to the risk of pathogen evolution. Our final goal is to illustrate how this framework may be used to develop resistance-breeding strategies that aim to attain durable disease resistance.

An important theme running through this paper will be the concept of the genetic structure of populations. We define the genetic structure for a species as the amount and distribution of genetic variation within and among populations of that species. The genetic structure of an individual population within a species is determined by the evolutionary history of that population. Genetic structure is a consequence of the interactions among the five factors that affect the evolution of populations. The genetic structure of individual pathogen populations can vary through time and space as these populations evolve, or adapt,

in response to local environmental changes. But the overall genetic structure of a species is not likely to change over human time scales, except in rare cases such as the recent emergence of sexual populations of the potato late blight pathogen *Phytophthora infestans* outside of Mexico.

## Boom and bust cycles: our current understanding

Plant pathologists have witnessed changes in pathogen populations at least hundreds of times over the nearly hundred years since Biffen first described the Mendelian inheritance of major resistance genes (Biffen, 1905). The most dramatic changes were those that accompanied the breakdown of major resistance genes. Boom and bust cycles have been documented most thoroughly with regards to cereal rusts (McIntosh et al., 1983; Samborski, 1985; Martens & Dyck, 1989; Hulbert et al., 1991; Johnson, 1992; Kolmer, 1992) and powdery mildews of cereals (Wolfe, 1984; Brown et al., 1993, Wolfe & McDermott, 1994; Brown et al., 1997) that exhibit a gene-for-gene (GFG) interaction with their hosts (Flor, 1956). In the majority of these cases, a single resistance gene with large effect became widely distributed over a large geographical area because it was effective against a large fraction of the pathogen population (the 'boom'). The pathogen population adapted to this new environment (the presence of a major resistance gene) by evolving a new population that could overcome this resistance gene (the 'bust'). In these cases, the 'breakdown' of genetic resistance was not due to a mutation in the resistance gene of the host. Rather, it was due to the evolution of the local pathogen population as a result of selection for mutants, recombinants, or immigrants that were better adapted to the resistant variety.

In our current understanding of the GFG interaction between plants and pathogens, pathogens produce elicitor molecules (the products of avirulence genes) that are recognized by specific receptors (products of resistance genes) in the plant. When a plant cell receptor recognizes a pathogen elicitor, a hypersensitive response is activated that leads to death of the infected plant cell as well as the pathogen. Mutations from avirulence to virulence in the pathogen lead to a change in the elicitor that causes non-recognition by the host receptor. Under this GFG model, a breakdown in resistance is due to an increase in the frequency of pathogen strains that harbor a mutation from avirulence to virulence. Virulent mutants increase in frequency because host defence systems are not activated early enough to prevent infection and subsequent pathogen reproduction. After the virulent mutation has reached a detectable frequency (which can be as low as 1%) in the pathogen population, the resistance gene is no longer considered effective, and we say that its resistance is 'broken'. GFG resistance is often called major-gene resistance, race-specific resistance, or vertical resistance because its effects are large and effective only against the portion of the pathogen population that produces the elicitor.

Resistance in plants can also be due to other types of genetically encoded products, such as phytoalexins, PR-proteins, chitinases, and modifiers of host defence responses. These resistance genes may operate alone or in combination to affect the degree of resistance displayed by the plant. We consider it likely that the individual actions of these genes are small and additive, leading to a quantitative resistance response that differs in inheritance and in mode of action from a GFG interaction. These quantitative responses are sometimes called minor-gene resistance, partial resistance, quantitative resistance or horizontal resistance and they generally do not follow the GFG pattern of the boom and bust cycle. Minor resistance genes rarely provide the total resistance observed in GFG resistance, but they tend to be equally effective against all strains of a pathogen population, even those which do not produce elicitors. We believe that the population genetic processes that will be described here also apply to minor resistance genes, but we will not treat them explicitly in this paper.

In order to fully comprehend the process that leads to the breakdown of a resistance gene, we need to better understand the processes that govern pathogen evolution. In the next section we will briefly illustrate how the five evolutionary forces operate individually and then interact to affect the durability of major gene resistance. From this understanding, we then will develop a simplified model that can be used to evaluate the 'risk' that a pathogen will evolve to overcome major resistance genes. Finally, we will use this model to propose a breeding strategy that we consider most likely to break the boom and bust cycle and lead to durable resistance.

## The five evolutionary forces

At this point, we offer a brief overview of the five evolutionary forces considered by population geneticists

involved in evolutionary studies of plant pathosystems. We will be paying particular attention to their effect on major resistance genes and the durability of major gene resistance. The five evolutionary forces are mutation, genetic drift, gene flow, reproduction/mating system, and selection.

Mutation is the ultimate source of genetic variation, directly leading to changes in the DNA sequence of an individual gene and thus creating new alleles in populations. Populations with more alleles have greater gene diversity than populations with few alleles. Mutation is the process that creates new virulent strains of plant pathogens that break major gene resistance. Mutation also creates strains with increased virulence or aggressiveness that can erode minor gene or quantitative resistance. Under our current understanding of the GFG interaction, a mutation in the avirulence allele, which encodes the elicitor recognized by a resistance gene, is needed to create a virulent pathogen strain. Mutations from avirulence to virulence are rare and operating in isolation would not cause a breakdown in resistance. But when mutation is coupled with efficient directional selection (i.e. widespread deployment of a resistance gene), virulent mutants increase in frequency rapidly and cause a resistance gene to lose its effectiveness. Pathogen populations with an active transposable element may exhibit higher mutation rates than populations without active transposons. Though it is difficult to imagine how a disease management program could reduce mutation rates and thus limit the creation of new alleles, any activity that slows the movement of active transposable elements or genome rearrangements among populations could potentially affect overall mutation rates for a pathogen species.

Population size affects the probability that mutants will be present, and also can influence the diversity of genes in a population through a process called random genetic drift. Large populations have more mutants than small populations because mutation rates are relatively constant and usually quite low. Thus, large populations are expected to have greater gene diversity (more alleles) than smaller populations. Genetic drift occurs when a small, randomly chosen subset of a population survives a catastrophic event that causes a severe reduction in population size (a bottleneck) or when a small, random subset of a pathogen population colonizes a new host population (founder event). The frequency of mutant alleles in the surviving or founding population can differ significantly from the frequency of the mutant alleles in the original population. Founder events often occur in plant pathology when a disease is introduced into a new area by accident or as a result of a breach of quarantine. Any disease management program that keeps pathogen population sizes small assists control by limiting the gene diversity in the pathogen population.

Gene flow is a process in which particular alleles (genes) or individuals (genotypes) are exchanged among geographically separated populations. For strictly asexual organisms that do not recombine particular genes with the recipient population, entire genotypes are exchanged among populations and we will refer to this process as genotype flow. To illustrate the effects of gene flow, imagine that each farmer's field maintains a discrete pathogen population. If these discrete pathogen populations exchange many propagules, then these field populations become linked through gene or genotype flow, and evolve as a single, large unit though they are spatially distinct. Thus, gene flow can substantially increase population size by increasing the size of the 'genetic neighborhood' over which genes or genotypes are exchanged. Pathogens that exhibit a high degree of gene/genotype flow are expected to have greater genetic diversity than pathogens with low degrees of gene/genotype flow because high gene flow pathogens have a larger effective population size. Pathogens that produce propagules with the potential for long distance dispersal, such as rusts and *Blumeria graminis*, tend to have large genetic neighborhoods, which may encompass entire continents (e.g. the Puccinia pathway in North America). Pathogens with propagules that move only short distances may exist in relatively small genetic neighborhoods, which may encompass only one field or even one section in a field (e.g. nematodes and soilborne pathogens such as *Phytophthora sojae* and *Armillaria gallica*). It is clear that the size of the genetic neighborhood is affected by the method of natural dispersal of pathogen propagules. It also is affected by anthropogenic activities. Humans move many pathogens beyond their natural dispersal limits as a result of intercontinental travel and global commerce. Gene/genotype flow is the process that moves newly arisen virulent mutant alleles among individual field populations. A dramatic example of genotype flow was the global movement of one or a few clones of *Phytophthora infestans* from Mexico to the USA in 1843 (Stevens, 1933), then to Europe in 1845 (Bourke, 1964), and then around the world in the 1850s (Goodwin et al., 1994). More recent examples of genotype flow are the movement of *Fusarium oxysporum* f.

sp. *cubense* clones among banana plantations (Koenig et al., 1997). Any management tactic that limits movements of genes and genotypes among pathogen populations limits the spread of mutant alleles and genotypes.

Reproduction system and mating system will affect the way that gene diversity is distributed within and among individuals in a population, leading to different degrees of genotype diversity. Reproduction can be either sexual or asexual (parthenogenic for nematodes). Reproduction also can be mixed, comprising a mixture of sexual and asexual reproduction as occurs for many fungi. Mating system is relevant only to the sexual component of reproduction, and it can vary from strict inbreeding to obligate outcrossing. Some pathogens (e.g. all *Fusarium oxysporum* formae speciales and most bacteria) appear to reproduce only asexually in agroecosystems. These pathogens exist as a series of discrete clones or clonal lineages with little evidence for recombination among clonal lineages. Populations of these pathogens usually exhibit a low level of genotype diversity. Variation within the clonal lineage occurs as a result of mutation. For example, a new virulent mutant of a Fusarium oxysporum wilt pathogen (a new pathotype) may emerge as a result of a mutation in a single clonal lineage. In strictly asexual pathogens, measures of genotype diversity are more meaningful than measures of gene diversity because most of the genetic diversity is distributed among the clonal lineages. At the other extreme of reproduction are pathogens such as smuts that must undergo a sexual cycle in order to form an infectious dikaryon and reproduce. An advantage of sex to the pathogen is that new combinations of genes can come together through recombination each generation, leading to a high degree of genotype diversity that may enable some component of the pathogen population to survive in a threatening environment. A less advantageous result is that especially fit combinations of genes (known as coadapted gene complexes) are broken up through recombination each generation, so it becomes difficult to maintain groups of alleles that offer an advantage in specific environments. Populations of sexual pathogens usually exhibit a high degree of genotype diversity, so measures of gene diversity are needed to compare populations. If a mutation to virulence occurs during one growing season, the mutation will be recombined into many different genetic backgrounds by the next growing season. An exception to these expectations occurs for pathogens that undergo extreme inbreeding such as the sorghum head smut pathogen, *Sporisorium reilianum* (Torres-Montalvo, 1998). In these cases, many generations of inbreeding can produce a genetic structure composed of a series of clonal lineages, which more closely resembles the expected genetic structure of asexual organisms.

Many of the most damaging and most dangerous pathogens undergo a combination of sexual and asexual reproduction. We will refer to these as 'mixed' reproduction systems. Pathogens with mixed reproduction systems have significant advantages over strictly asexual or strictly sexual pathogens. During the sexual cycle, many new combinations of alleles (genotypes) are produced which can be 'tested' in different environments, including the presence of new resistance genes, fungicides, or antibiotics. During the asexual reproduction phase, combinations of alleles (genotypes) that are most fit are held together through clonal reproduction and may increase to a high frequency. Thus these pathogens can exhibit a high level of both gene and genotype diversity. The spatial and temporal distribution of clones or clonal lineages within and among populations will depend mainly on the dispersal potential of the asexual propagules. If the asexual spore or propagule is capable of long-distance dispersal, then the clone with highest fitness can become distributed over a wide area through genotype flow relatively quickly, causing an obvious epidemic. This process used to occur for *Puccinia graminis* f. sp. *tritici* in North America (pre-barberry eradication) and still occurs for many powdery mildews. If the asexual propagule has limited dispersal potential (e.g. splash-dispersed conidia), then the most damaging clones may not become widespread and the epidemic may be limited to a 'hot-spot' that is only a few meters in diameter in a field. As we will describe later, this appears to be the case for *Mycosphaerella graminicola* on wheat and for *Rhynchosporium secalis* on barley.

The ability of the asexual spores or vegetative clones to persist through time can have a large impact on the genetic structure of pathogen populations. For many pathogens, the sexual fruiting bodies and sexual spores are the over-seasoning survival structures. In these cases, it is likely that particular clones will not persist through time and the pathogen population will be composed of a new series of genotypes at the start of each growing season. However, if asexual spores or other propagules can persist across seasons, and some clones exhibit a high degree of fitness, then a pathogen population may have a clonal genetic structure even though sexual recombination occurs every year (An-

derson & Kohn, 1995). Management strategies that limit the occurrence of sexual reproduction or that limit the persistence and spread of asexual propagules assist with control.

The final evolutionary force, selection, has been the most studied and is probably the force that is most easily managed in agroecosystems. Selection is the main force that drives changes in frequencies of mutant alleles. The strong, directional selection that occurs when a major resistance gene (receptor) becomes widely distributed over a large geographical area drives the increase in frequency of the virulent mutant that has lost the elicitor (avirulence allele), until the resistance gene is broken. The many examples of broken resistance genes offer abundant evidence that selection is efficient in most modern agricultural ecosystems that are based on monoculture and genetic uniformity. The rate of change in frequency of an allele under selection is usually expressed in terms of a parameter known as a selection coefficient (s), which is a measure of fitness relative to a defined genotype, usually chosen as the most fit genotype in the population under consideration. Measures of s have varied widely, depending on the plant pathosystem under study and the way in which it is measured. But in most careful studies, s ranges from 0.025 to 0.622 (Ennos & McConnell, 1995), suggesting that selection can rapidly change the frequencies of virulence alleles under the strong, directional selection that occurs when single major resistance genes are deployed one at a time.

But there are several other possibilities for deploying major resistance genes that can change the way that selection operates on the pathogen population (Figure 1). The most common alternative is to 'pyramid' several major resistance genes into a single cultivar in the hope that the pathogen will not be able to undergo a sequence of mutations corresponding to each resistance gene. Another option is to generate disruptive selection by rotating genes through time and space or by growing mixtures of cultivars with different resistance genes. These strategies disrupt directional selection by favouring different mutant alleles or genotypes at different times and places, reducing the rate at which the mutant allele or genotype increases in frequency. Gene rotations and mixtures have not yet been widely explored or exploited in agroecosystems, though some preliminary attempts show great promise against some of the most notorious plant pathogens (Browning & Frey, 1969; Wolfe, 1985; Zhu et al., 2000).

## Interactions among the evolutionary forces determine genetic structure

Interactions among the five evolutionary forces ultimately determine the genetic structure of pathogen populations, and hence the evolutionary potential of these populations. For example, mutation may constantly produce mutant alleles at the avirulence locus, but in the absence of a matching plant receptor (R-gene) and corresponding directional selection, the virulence allele may never increase to a detectable frequency. Similarly, virulent mutants that originate and increase in frequency during a growing season in a field planted to a resistant cultivar may never cause a widespread epidemic if gene flow among fields is very low as a result of a steep dispersal gradient and effective quarantines. And highly fit genotypes that are distributed over long distances may never become established in some locations because they experience regular local extinctions as a result of bottlenecks imposed by local climatic conditions (e.g. cereal rusts in the Canadian prairies). The end result of the interactions among the five evolutionary factors is the observed genetic structure of the pathogen population. By using selectively neutral genetic markers and hierarchical sampling to determine the genetic structure of pathogen populations, we can begin to understand the evolutionary forces that shaped these populations, and infer the importance of the individual evolutionary factors (McDonald, 1997). At this point, it will be useful to differentiate between the two types of genetic diversity that are components of genetic structure, gene diversity and genotype diversity.

Gene diversity refers to the number and frequencies of alleles at individual loci in a population. Gene diversity increases as the number of alleles increases and the relative frequencies of those alleles becomes more equal. For example, a locus that has three alleles is more diverse than a locus with two alleles. For selectively neutral loci, gene diversity is affected by population size, the age of the population, and gene flow. Large populations have more alleles than small populations as explained earlier. Older populations have more alleles than young populations because there have been more generations in old populations for mutation to occur and then for genetic drift to increase these alleles to a detectable frequency. Thus, populations at the center of origin of a species generally have the greatest gene diversity. High gene flow also increases gene diversity because gene flow intro-

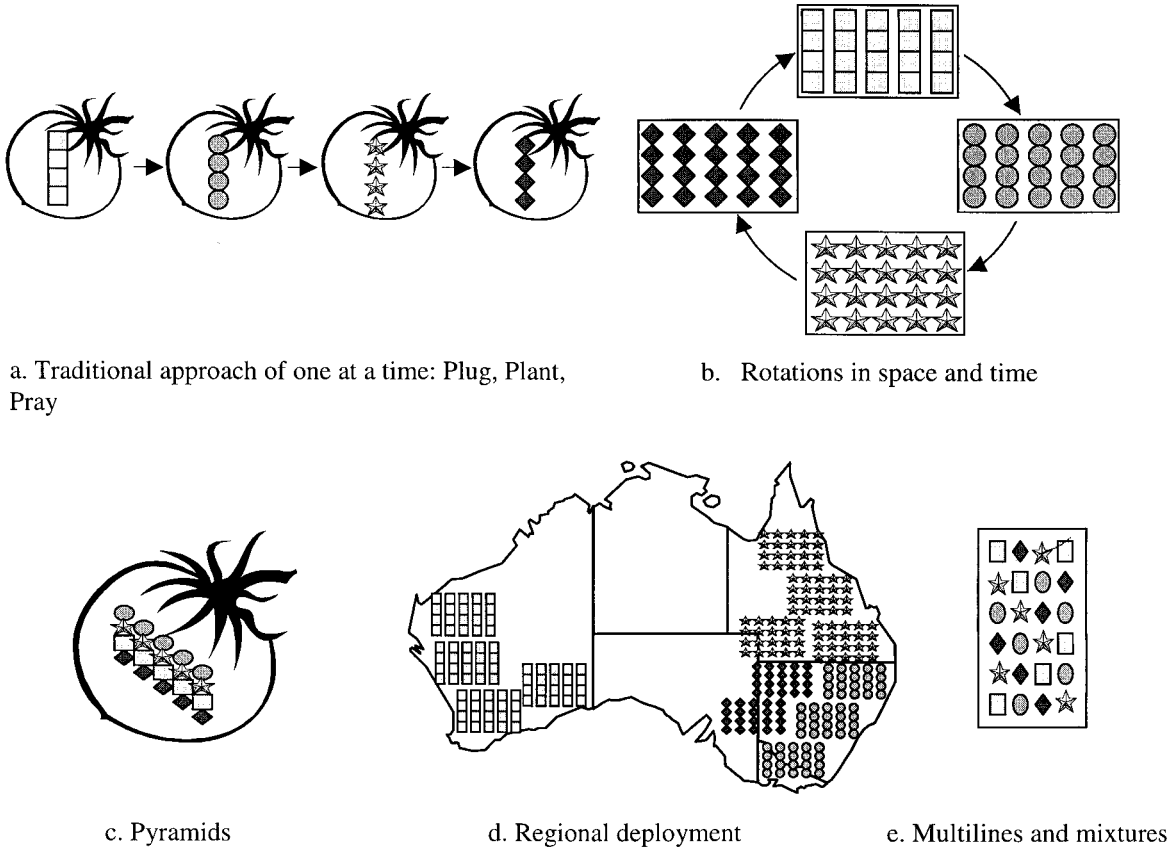


*Figure 1.* Strategies for deployment of major resistance genes (R-genes). A. The traditional 'plug, plant, and pray' approach of deploying single R-genes one at a time in a sequence that matches the boom and bust cycle. B. Rotations of R-genes in time or space. Each R-gene is deployed over a limited number of years or area, and is withdrawn before the corresponding virulence allele achieves a high frequency in the pathogen population. C. R-gene pyramid. All R-genes are placed together in one plant genotype. D. Regional deployment. Different R-genes are grown in different regions. This can be part of a gene rotation as described in panel B. E. Cultivar mixtures and multilines. Individual R-genes are grown as an intimate mixture in the same field.

duces new alleles into populations that are part of the same genetic neighborhood.

Genotype diversity refers to the number and frequencies of multilocus genotypes, or genetically distinct individuals, in a population. Genotype diversity is a meaningless concept for eucaryotes like humans that reproduce by sexual outcrossing because each individual in a population is unique (except for rare identical twins). But it becomes very important for plant pathogens that have a significant component of asexual reproduction in their life history. Genotype diversity is affected by reproduction/mating system, selection, genetic drift, and genotype flow. Pathogens that undergo regular sexual reproduction are expected to have more genotype diversity than pathogens that are asexual. The number and frequencies of clones in a population can fluctuate as a result of directional selection that favors particular clones, or as a result of genetic drift (bottlenecks and founder events). New genotypes may be introduced into some populations as a result of genotype flow.

We propose to apply this knowledge of the evolutionary forces and pathogen genetic structure to make predictions regarding the relative risks posed by different pathogens for breaking down resistance genes. In the next sections, we will develop a broad framework in which to assess the relative risk posed by different pathogens, and then use this framework to develop complementary guidelines to determine the best strategy for resistance breeding. Our hypothesis

is that much of the durability of resistance genes is due to the nature of the pathogen population rather than exclusively to the nature of the resistance gene that is used. It is obvious from the outset there will be some exceptions where the nature of the resistance gene plays a key role in durability, e.g. mlo resistance in barley has been durable though *Blumeria graminis* f. sp. *hordei* has a high evolutionary potential according to our risk model. Though it is likely that other exceptions will emerge, we offer these guidelines in anticipation that the general pattern will hold true for the majority of pathogens. The framework we will offer can be used as a hypothesis to test against a large number of plant pathosystems in addition to the ones mentioned in this paper. The underlying principles of the framework can be tested individually or in combination according to the available knowledge of the population genetics of any pathogen under consideration.

## Risk assessment: the evolutionary potential of plant pathogens

In this section, we briefly summarize and rank the risks inherent for each of the five evolutionary forces, with a goal of developing simple guidelines that can be used to evaluate the evolutionary potential of different pathogens according to their genetic structure. We propose here that the pathogens with the greatest evolutionary potential offer the greatest 'risk' of breaking down major resistance genes or evolving to counteract other control methods such as applications of pesticides or antibiotics.

Pathogens with high mutation rates present a greater risk than pathogens with low mutation rates because a high mutation rate increases the likelihood that the mutation from avirulence to virulence will be present in a pathogen population. Mutation rates are generally low, though they can differ among loci and pathogens. Pathogen populations with active transposable elements may pose a greater risk than populations without active transposable elements. But mutation operating in isolation from other evolutionary forces is unlikely to occur at a high enough rate to produce detectable changes in allele frequencies.

Pathogens with large population sizes have more evolutionary potential than pathogens with small population sizes because more mutant alleles are present in the large populations. Pathogens that undergo regular, severe reductions in population size, e.g. as a result of crop rotations or annual climatic extremes that kill the majority of individuals, are less diverse and slower to adapt than populations that maintain a high population size year round. An example is the cereal mildew pathogen *Blumeria graminis* that can maintain a large population size in winter on winter cereal crops, and in summer on spring cereal crops, avoiding bottlenecks that would reduce gene and genotype diversity. On the other hand, the elimination of the barberry alternative host of *P. graminis* f. sp. *tritici* between 1920–1950 (Roelfs & Groth, 1980; Burdon & Roelfs, 1985), significantly reduced the effective population size of the pathogen and reduced both gene and genotype diversity. Crown rust on oats (*Puccinia coronata* f. sp. *avenae*) in Australia maintains its population size throughout the year by over wintering on wild oats species (Park et al., 2000). Two simple ways to minimize pathogen population size are by using regular crop rotations and by avoiding the cultivation of extremely susceptible varieties that allow pathogen population size to increase explosively.

Pathogens with high gene flow pose a greater risk than pathogens with low gene flow for two reasons. 1) High gene flow populations have larger effective population sizes, and thus maintain more alleles. 2) High gene flow pathogens are more likely to transmit virulent mutants across a large geographical area. We hypothesize that gene flow involving asexual propagules (genotype flow) poses greater risks than movement of sexual propagules (gene flow) because the asexual propagule represents a linked package of coadapted genes that was pre-selected for a high level of fitness in the crop environment where it originated. Sexual propagules (e.g. ascospores) represent new combinations of alleles that have not yet been tested in any environment. The control objective here is to limit the extent of gene flow among pathogen populations. While we cannot affect 'natural' dispersal of pathogen propagules by wind, water, or insects, we can limit the potential for long distance dispersal aided by man, including movement of infected plant material, soil, or contaminated equipment among otherwise isolated pathogen populations. We can also attempt to eliminate living 'bridges' of susceptible host materials that can act as stepping stones between populations, or plant strips of resistant plants (or non-host plants) to act as barriers between adjacent susceptible host populations.

Pathogens that undergo regular recombination (this can include processes distinct from meiosis such as bacterial conjugation, recombination between viral

genomes in plants with mixed infections, and hyphal anastomosis and/or parasexual recombination in fungi) pose higher risks than pathogens that undergo no or little recombination. Recombination allows new combinations of alleles to come together and be tested against new environments. A pathogen that undergoes regular recombination can put together new combinations of mutant alleles (e.g. virulence alleles) as rapidly as breeders can recombine major resistance genes. For this reason, resistance gene pyramids may not be an effective long-term breeding strategy against pathogens that undergo regular recombination. We cannot alter a pathogen mating system directly, but we can take steps to modify its effectiveness by altering ratios of mating types, by eliminating alternate hosts that are needed for sexual reproduction, or by modifying the environment to make it unfavorable for formation of the sexual stage. Intervention in the latter case could involve cultural practices such as burying stubble or other plant debris where the sexual stage may occur. This would be useful in the *Leptosphaeria maculans* – oilseed rape pathosystem, where the primary inoculum consists of ascospores from residues of mainly crucifer hosts (Hall, 1992).

For pathogens that undergo regular recombination through meiosis, pathogens that outcross pose a greater risk than inbreeding pathogens because more new genotypes are created through outcrossing. This hypothesis is tempered by the realization that inbreeding also offers some advantages to pathogens, because regular inbreeding, like asexual reproduction, tends to produce clonal lineages with linked sets of alleles that may form a coadapted gene complex. Thus pathogen populations such as smuts that undergo regular cycles of inbreeding may develop a very fit set of coadapted alleles that remains together and becomes widely disseminated.

Pathogens with mixed reproduction systems that include both sexual and asexual reproduction pose the highest risk of evolution because they receive benefits from both styles of reproduction. Sexual recombination allows many new combinations of alleles to come together and then be tested in the local environment. Asexual reproduction allows the most fit genotype to reproduce as a clone, keeping together a fit combination of alleles and making it possible for this allele combination to become widely distributed if the asexual propagules are dispersed over long distances.

Pathogen populations that are exposed to strong, directional selection over many generations pose a greater risk than populations that are exposed to weaker selection due to partial resistance or to disruptive selection due to temporal or spatial patterning of the selective force. Selection is the evolutionary force that is most easily manipulated by humans, and thus offers the most practical point for intervention in the evolutionary process. Agroecosystems based on widespread deployment of single, major resistance genes (genetically uniform monoculture) place strong directional selection on the pathogen population. Agroecosystems that deploy major resistance genes in mixtures, or in rotations through time and space will reduce the efficiency of selection, or impose stabilizing or disruptive selection that can slow the rate of increase in the frequency of virulent mutants. We summarized the contributions of each evolutionary force to the assessment of risk in Table 1.

## A simplified diagram for risk assessment

Figure 2 is a simplified diagram that we propose as a model for assessing the evolutionary risk posed by most plant pathogens. The format used to present the risk categories was inspired by a similar figure presented by Brent & Hollomon (1998) (Figure 2 in Brent & Holloman), in a FRAC publication that considered the risk of evolution of fungicide resistance. Figure 2 considers only the evolutionary risk due to differences in reproduction/mating system and gene flow. Mutation rate was not included in our risk assessment because we assumed that mutation rates would be low and relatively constant across all pathogens, so the differences in this parameter for different pathogens on average are expected to be relatively small. For pathogens known to experience very high mutation rates, these risk values can be increased. Selection was not included under the assumption that selection is likely to be efficient in the genetically uniform monocultures that dominate modern agricultural ecosystems so that mutants will increase in frequency quickly if a selective influence is present. As we explained earlier, selection risk can be adjusted by modifying the environment through different strategies of resistance gene deployment, such as gene rotations or mixtures. Population size was not included under the assumption that most pathogen populations are very large, so it is likely that virulent mutants will be present and the effects of genetic drift will be small. To modify the risk assessment due to extremely large or extremely small pathogen populations, we suggest modifying the risk values in Figure 2 as follows. Mul-

*Table 1.* Extremes of evolutionary risk posed by plant pathogens and example factors that affect risks

| Highest risk of evolution | Lowest risk of evolution |
|---|---|
| High mutation rate<br>transposable elements | Low mutation rate<br>no transposons |
| Large population sizes<br>large overseasoning population<br>local extinction rare<br>no genetic drift | Small population sizes<br>no overseasoning propagules<br>extinction of local populations common<br>significant genetic drift |
| High gene flow<br>asexual propagules dispersed by air over long distances<br>human-mediated long distance movement | Low gene flow<br>asexual propagules soil-borne |
| Mixed reproduction system<br>regular sexual outcrossing and asexual spores produced | Asexual reproduction<br>only asexual spores or other asexual propagules produced |
| Efficient directional selection<br>R-gene deployed in genetically uniform monocultures | Disruptive selection<br>R-genes deployed in mixtures<br>R-genes deployed as rotations in time or space |

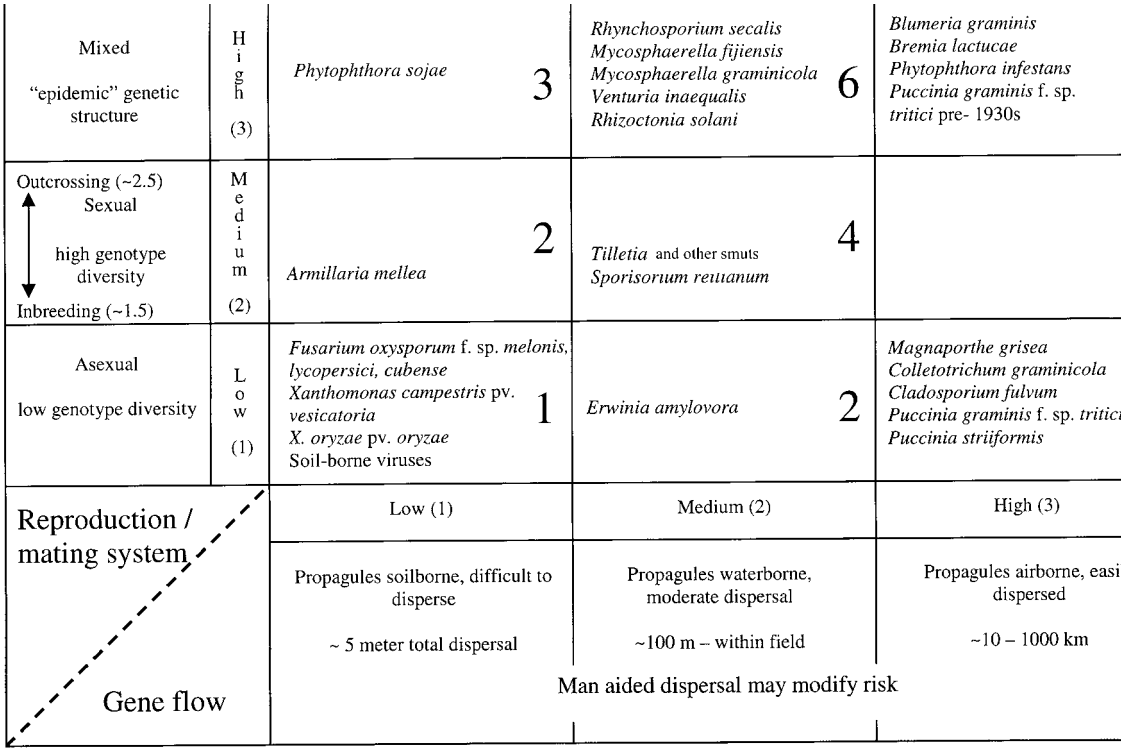


*Figure 2.* Scale of evolutionary risk ranked according to reproduction/mating system and gene flow potential. The organization of this diagram is modified from Figure 2 of Brent & Holloman (1998). This model assumes that mutation rates are constant and that selection is efficient for all pathogens. If overseasoning population sizes are very large, multiply risk by 1.5. If overseasoning population sizes are very small, multiply risk by 0.5. Placement of example pathogens is explained in text.

tiply the risk value by 0.5 if the population size is very small (pathogen population experiences regular bottlenecks and has short-lived overseasoning propagules), and by 1.5 if the population size is extremely large (population experiences year round multiplication, is capable of rapid reproduction, and produces long-lived overseasoning propagules). Finally, it is important to recognize that these proposed risk categories might need to be adjusted on a case-by-case basis as a result of anthropogenic activities. For example, gene flow may be increased beyond the normal biological limits of spore dispersal by movement of inoculum through international commerce and travel. Any activities that reduce the effectiveness of quarantines, such as smuggling or war, may lead to an increase in gene flow. Similarly, the amount of sexual reproduction may be affected by removal of alternate hosts or by changes in cultivation or sanitation practices.

The risk values presented in Figure 2 are on a 1–9 scale. These numbers are unitless and have no specific biological meaning. The numbers represent an initial attempt to provide a relative ranking of the evolutionary potential inherent in different pathogen life histories. The present ranking system assumes that reproduction/mating system and gene flow affect evolutionary potential equally, and that these effects are multiplicative. As more knowledge accumulates and these hypotheses are tested against many different pathosystems, we may learn that these forces do not contribute equally to evolutionary potential or that their effects are additive rather than multiplicative. But the proposed scale offers many possibilities for assigning relative evolutionary risk. For example, pathogens that have exclusively asexual reproduction and little potential for gene flow are assigned to the lowest risk category. This category includes some bacterial pathogens and the *Fusarium oxysporum* formae speciales. At the other extreme, pathogens that have mixed reproduction, including an annual outcrossing sexual cycle and asexual spores that are disseminated over long distances by wind are assigned to the highest risk category. This category includes pathogens such as the powdery mildews. In the intermediate risk categories are pathogens that we expect to have more limited evolutionary potential as a result of lack of an asexual propagule that has high gene flow potential, or lack of regular outcrossing that produces new recombinants.

Figure 2 should be considered a preliminary guide for assigning risk. It is much too simplified to be used as an exclusive decision-making tool. The primary function of Figure 2 is to guide thinking about the evolutionary potential of different plant pathogens and to illustrate in very general terms the possible interactions that may occur among the evolutionary forces as a result of pathogen life history. We believe it also offers a very flexible framework that can be used to develop testable hypotheses about the evolutionary forces driving pathogen evolution. For example, Figure 2 hypothesizes that pathogens with a regular sexual cycle will evolve faster than pathogens without a mechanism for recombination. It also hypothesizes that pathogens that produce asexual propagules that are distributed over long distances will break down resistance genes faster than pathogens with short distance dispersal of asexual propagules. At this point in time, a thorough knowledge of population genetic structure is not available for most pathogens. But as this knowledge continues to develop, we hope that Figure 2 will offer testable hypotheses that correlate pathogen life history with pathogen evolutionary potential. Our challenge is to add a large number of plant pathogens to this risk model and determine if the observed evolutionary rate of pathogens correlates with the predicted evolutionary potential. The main limitations to testing the model at this point are that adequate population genetic data are available for relatively few plant pathogens and that the rate of breakdown of resistance genes often is not recorded in the scientific literature. In the next section, we will present several examples showing how different pathosystems can be fit into this risk diagram. In the final section of this paper, we will propose guidelines to use this risk diagram to design the most efficient resistance breeding strategy.

## Example plant pathosystems

Over the last decade, our lab has characterized the genetic structures of several pathogen populations on a variety of cereals. These examples are especially useful for comparing the genetic structures of different pathogens because the same methodology was used in each case. For each pathogen, we used from 6-12 individual RFLP genetic markers usually combined with RFLP fingerprints to characterize fungal populations that were collected using a hierarchical sampling strategy from geographically defined populations (usually a farmer's field) for crops grown over a large geographical area. And the same analytical methods were used to determine the spatial scale of genetic variation, the mating and reproduc-

*Table 2.* Example risk assessments and proposed breeding strategies for plant pathosystems described in main text. MGR = Major Gene Resistance; QR = Quantitative Resistance

| Pathogen | Population size | Mating/Reproduction system | Gene flow | Risk category | Proposed breeding strategy |
|---|---|---|---|---|---|
| Asexual rusts | Medium | Asexual | High | 3 | MGR pyramids |
| *Colletotrichum graminicola* | Medium | Asexual | High | 3 | MGR pyramids |
| *Fusarium oxysporum* f. sp. | Medium | Asexual | Low | 1 | Single MGR |
| *Mycosphaerella graminicola* | Large | Mixed | High (ascospores) | 6 | QR, MGR mixtures and multilines |
| *Phaeosphaeria nodorum* | Medium/Large | Mixed | High (ascospores) | 6 | QR, MGR mixtures and multilines |
| *Phytophthora infestans* (new populations) | Small/Medium (oospores) | Mixed | High | 9 | QR, MGR mixtures and multilines |
| *Phytophthora infestans* (old populations) | Small (founder event) | Asexual | High | 3 | MGR pyramids |
| *Rhizoctonia solani* | Medium | Mixed | Moderate | 6 | QR, MGR mixtures and multilines |
| *Rhynchosporium secalis* | Medium | Mixed? | Moderate | 6 | QR, MGR mixtures and multilines |
| *Sporisorium reilianum* | Small/Medium | Sexual, inbreeding | Moderate | 4 | Single MGR, MGR pyramids |

tion system, and to determine the degree of genetic similarity among field populations. Table 2 presents a summary of the evolutionary risk associated with these pathogens, and a proposed resistance breeding strategy.

### *Mycosphaerella graminicola* and *Phaeosphaeria nodorum* on wheat

*Mycosphaerella graminicola* and *Phaeosphaeria nodorum* are the two fungi that cause the Septoria diseases on wheat. Our lab has examined over 5000 isolates of *M. graminicola* and nearly 1000 isolates of *P. nodorum* from field populations around the world (McDonald et al., 1995; Keller et al., 1997; McDonald et al., 1999). These two fungi both have a mixed reproduction system, and both exhibit a genetic structure with a high degree of gene and genotype diversity distributed on a small spatial scale. For both fungi, practically every lesion on a leaf contains a genetically unique individual, and clones are not widely distributed within or among fields. When clones are identified, they tend to originate from the same 1 $m^2$ area of a field. Yet field populations from five different continents have the same RFLP alleles at similar frequencies. This genetic structure suggests that these pathogens exist as large populations that undergo regular sexual recombination, with asexual spores that move only a few meters over the course of a season (Chen & McDonald, 1996). The similarity in allele frequencies over large geographical distances suggests that gene flow has been a significant unifying force through time. The finding that populations are stable over time combined with the large number of alleles found at individual RFLP loci suggest that populations size are large for these fungi (Chen et al., 1994). We place both of these pathogens in risk category

6 because they both have mixed reproduction/mating systems and significant gene flow, but the asexual spores are not dispersed over long distances.

### *Colletotrichum graminicola* on sorghum

*Colletotrichum graminicola* causes the anthracnose disease on sorghum. Our lab examined 1278 isolates sampled from fields in Texas, Georgia, Honduras, and Zambia (Rosewich, 1996). No sexual stage is known for this pathogen, and it is thought to reproduce only asexually. This pathogen exhibited a clear clonal genetic structure in farmer's fields, with only one or a few multilocus genotypes found in each field. One clonal lineage predominated in a collection originating from a 250 km transect through Texas sorghum fields. And three other clonal lineages predominated in a disease nursery in Georgia over a three year period (Rosewich et al., 1998). One clone was shared between Georgia, Honduras, and Zambia. These findings suggest that genotype flow may be significant over large geographical scales, perhaps due to movement of the pathogen in contaminated seed. Greater genetic diversity was found in populations sampled from sorghum landraces in Honduras. We placed *C. graminicola* in risk category 3 because it has an asexual reproduction system and appears to have potential for long-distance genotype flow. But if genotype flow becomes restricted due to improved seed sanitation or quarantine restrictions, it would be placed in risk category 1.

### *Sporisorium reilianum* on sorghum

*Sporisorium reilianum* causes sorghum head smut. Our lab examined 459 isolates sampled from fields in Texas, Mexico, and Niger (Torres-Montalvo, 1998). This basidiomycete must form a dikaryon in order to infect its host and must undergo sexual reproduction to form a new generation of overwintering teliospores. Populations of this fungus sampled over a distance of 600 km from seven fields in Texas and Mexico were composed of a series of clonal lineages, with from 7 to 16 multilocus genotypes found in each field. But only two alleles were found for each RFLP locus. This genetic structure is consistent with a mating system characterized by regular inbreeding with occasional outcrossing. The low number of alleles found at each locus suggests that population sizes are generally small. There was a significant correlation between genetic distance and geographical distance among populations, suggesting that gene flow follows a stepping-stone model and that gene flow is significant on a regional basis (600 km), perhaps due to movement of infected seed. But the population from Niger was genetically distant from the Mexican and Texan populations, suggesting that gene flow is not intercontinental. We placed *S. reilianum* in risk category 4 because it has low genotype diversity as a result of regular inbreeding as well as low gene diversity and it appears to have limited potential for gene/genotype flow.

### *Rhizoctonia solani* on rice

*Rhizoctonia solani* AG-1 IA causes sheath blight on rice. Our lab assayed 182 isolates collected from six rice fields in Texas (Rosewich et al., 1999). The genetic properties of this basidiomycete have been difficult to determine due to a lack of adequate genetic markers, and it was not clear whether field isolates existed as homokaryons or heterokaryons or whether sexual reproduction played a role in the genetic structure of field populations. We found that gene diversity and genotype diversity were moderate in all populations for this pathogen, with only one shared multilocus genotype found at a significant frequency in three of the fields. This suggests that widespread clones are not a characteristic feature of the genetic structure of this pathogen, but that genotype flow does occur at detectable levels. The similarity in allele frequencies among populations suggested that sufficient gene flow occurred over the $\sim$300 km spatial scale sampled to prevent genetic drift. Analyses of multilocus associations suggested that sexual reproduction occurred in field populations, but that these populations were not random-mating. We placed *R. solani* in risk category 6 because of its mixed reproduction system and under the assumption that the asexual sclerotia which are assumed to be the units of asexual propagation offer only a moderate potential for gene flow on a regional basis.

### *Rhynchosporium secalis* on barley

The final example from our lab is *Rhynchosporium secalis*, which causes the barley scald disease. We

examined 543 isolates sampled from Australia, California, Finland, and Norway (Salamati et al., 2000). No teleomorph has been identified for this pathogen and it is generally assumed that the only form of reproduction is asexual. But field populations of *R. secalis* have a genetic structure remarkably similar to *M. graminicola* and *P. nodorum*, which undergo regular cycles of sexual reproduction. All field populations of *R. secalis* in our sample exhibited high levels of gene and genotype diversity, with no evidence for widespread clones past a spatial scale of two meters. The relatively large number of alleles per locus suggests that population sizes are large and the effects of genetic drift are small. Populations separated by long distances share the same alleles, but the allele frequencies are quite different, suggesting that gene flow is moderate regionally, but restricted between continents. We placed *R. secalis* in risk category 6 for the same reasons given for *M. graminicola* and *P. nodorum*, though it appears that *R. secalis* has less potential for intercontinental movement than the other two pathogens.

While it is beyond the scope of this paper to give an exhaustive list of example pathogen systems, we will include two additional examples that are common in the plant pathology literature and which may serve as good reference points in the risk diagram shown in Figure 2.

### *Phytophthora infestans* on potatoes

*Phytophthora infestans* causes late blight on potatoes and tomatoes. It is heterothallic and produces airborne asexual spores and therefore has a high potential for genotype flow. *P. infestans* first appeared in the USA in 1843 (Stevens, 1933) and migrated to Europe 1845 (Bourke, 1964). There is good evidence based on DNA fingerprints that the European population consisted of only one clone introduced from the USA (Goodwin et al., 1994). The introduced *P. infestans* population probably consisted of only the A1 mating type and thus was restricted to asexual reproduction (Goodwin, 1997). As a result of the limited number of founding individuals and lack of recombination, European populations of *P. infestans* have exhibited low levels of gene and genotype diversity over most of the 155 years since its introduction. The A2 mating type was first reported in Europe in 1980 (Hohl & Iselin, 1984), but it appears likely that the A2 was introduced into Europe some time in the 1970s. Since the introduction of the opposite mating type, *P. infestans* populations in Europe have begun to reproduce sexually and produce oospores that have the potential to overwinter in the soil. Thus, the introduction of the opposite mating type has increased the evolutionary potential in our risk diagram from category 3 to category 9 as a result of the introduction of a sexual cycle. It is not yet clear if the oospores will increase the population size sufficiently to merit a further increase in the risk value.

### *Fusarium oxysporum formae speciales* on many crops

*Fusarium oxysporum* formae speciales cause wilt diseases on many vegetable and ornamental crops, as well as in some field crops and plantation crops. None of these pathogens is known to undergo sexual recombination, and surveys of field populations with molecular markers suggest that most field populations are composed of a limited number of pathogenic clones. Though spores have limited potential for long distance movement, strains with the same VCG, pathotype, and DNA fingerprint often are found distributed over large geographical areas (Reviewed in Gordon & Martyn, 1997). In these cases, human activities have most likely moved clones over long distances. We place all *F. oxysporum* f. sp. in the lowest category on our risk scale, level 1, because of the strictly asexual reproduction, limited number of clones existing within fields, and the low potential for natural genotype flow among field populations. We recognize that genotype flow is likely to be much larger due to human commerce, but this could be controlled in theory through more effective quarantines and improved cultural practices.

## A decision diagram to aid resistance breeding programs

In the final section of this paper, we propose guidelines to apply knowledge of the evolutionary potential of the pathogen to decide how to choose appropriate types of resistance and how to deploy major resistance genes in a resistance-breeding program. The goal here is to offer advice to breeders who seek guidance on the best way to manage limited genetic resources in order to extend the useful life expectancy of available genetic resistance. We have tried to distill most of these ideas into a simple decision diagram that is shown in Figure 3. Our inspiration for this decision diagram

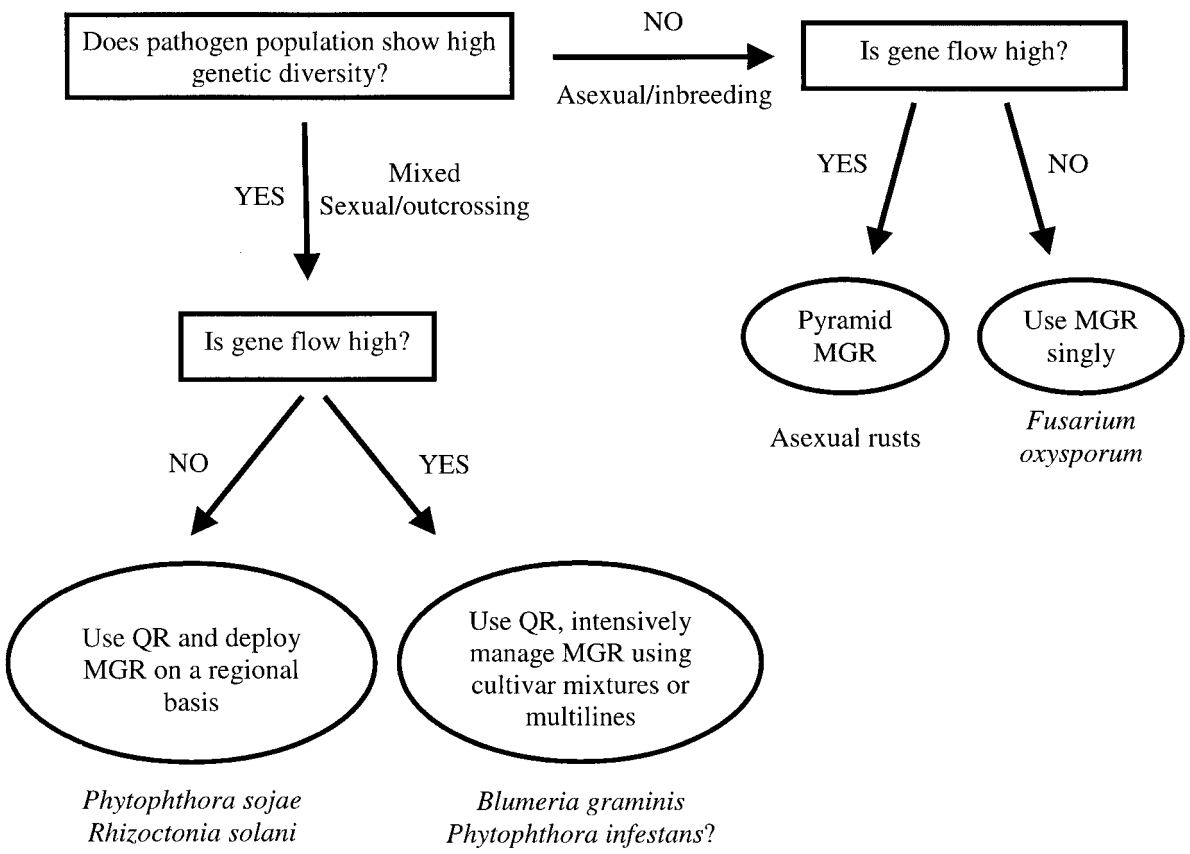


*Figure 3.* A simplified decision diagram to assist with developing resistance-breeding strategies to achieve durable disease resistance. MGR = Major Gene Resistance, resistance that has large effects, is based on the hypersensitive response and follows the receptor-elicitor model of the gene-for-gene interaction. QR = Quantitative Resistance, resistance that has small, nearly equal, and additive effects that are equally effective against all strains of the pathogen.

came from N.W. Simmonds (Edinburgh School of Agriculture) who published similar decision diagrams for tropical diseases, but without knowledge of the population genetics of the pathogen. The purpose of this diagram is to offer some broad guidelines to consider before embarking on a resistance-breeding project, with the objective of matching the weapons (R-genes) to the targets (pathogens). The goal is to choose the appropriate type of genetic resistance and then apply a resistance gene management strategy that will match the pathogen's biology and minimize the likelihood that the pathogen population will evolve to 'defeat' the resistance. In particular, we have attempted to indicate circumstances under which single, major gene resistance is likely to be most effective, when resistance gene pyramids are most likely to fail, and when gene deployment options such as cultivar mixtures or multilines should be considered early in the breeding program. A preliminary version of this decision diagram was published previously (McDonald, 1999).

The decision diagram assumes that major gene resistance (abbreviated MGR) is based on the receptor-elicitor model of the GFG and that minor gene resistance (abbreviated QR for 'quantitative resistance') is a quantitative character based on several unlinked genes that show equal and additive effects. The diagram assumes that both types of resistance are available to the breeder, and that major gene resistance is preferred because it is easier to recognize and is more easily introgressed into good agronomic types. To keep the diagram as simple as possible, the reproduction/mating systems of the pathogen are assumed to be either asexual/inbreeding, or mixed/outcrossing. Gene flow is assumed to be either high or low. This diagram can be extended with many additional branch points to encompass the full range of possible interactions among

evolutionary forces and potential pathogen population genetic structures. We did not attempt to include in this diagram the effects of different mutation rates, population sizes, or the full range of reproduction/mating systems. We decided to keep the diagram very simple in an effort to illustrate the general principles most clearly.

The outcome of the decision diagram is a general recommendation for choosing the type of resistance to use and the optimum deployment method with the aim of maximizing the useful life span of the resistance. This diagram should be considered in the appropriate context in a resistance-breeding program, as an aid to assist decision-making. It is not intended as an authoritative guide that is certain to lead to durable resistance.

At one end of the decision diagram are pathogens that have a strictly asexual reproduction system as well as a low potential for gene flow. In our risk model, these are pathogens with the lowest evolutionary potential, in risk category 1. These pathogen populations are characterized by low genotypic diversity that is often arrayed in a limited number of clonal lineages. When a mutation occurs from avirulence to virulence, it occurs randomly within a clonal lineage, and the virulent lineage does not disperse far from its origin due to lack of long-distance dispersal mechanisms. The virulence mutation is 'trapped' in the original clonal lineage due to the absence of meiosis and recombination. For these pathogens, a breeding strategy that relies on single major resistance genes is likely to be durable because the mutation to virulence will occur in a limited number of genetic backgrounds and the virulent lineages that inevitably arise are unlikely to move quickly to new fields planted to the same major resistance gene. An example of pathogens that follow this life history are the *Fusarium oxysporum* wilts on many crops.

The next category in the decision diagram is asexual pathogens that exhibit a high degree of genotype flow. These pathogens also exhibit low genotypic diversity, but when the virulent lineage arises by mutation, it is moved efficiently to neighboring fields or adjacent agricultural regions. In our risk model, these are pathogens with a relatively low evolutionary potential, posing a risk factor of 3. For these pathogens, a breeding strategy that pyramids major resistance genes is likely to be durable because it is unlikely that a sequence of multiple mutations to virulence (loss of several elicitors simultaneously) will occur in the same clonal lineage. An example of a pathogen that follows this life history is the wheat stem rust pathogen, *Puccinia graminis* f. sp. *tritici*, in North America following the removal of the barberry alternate host. Prior to the removal of barberry, *P. graminis* f. sp. *tritici* populations had a mixed reproduction system, and would have been placed in risk category 9. Other strictly asexual rusts also fall into this category. Pathogens that have a sexual cycle, but appear to be mainly inbreeding, such as *Sclerotinia sclerotiorum* (Kohli et al., 1995) and smuts such as *Sporisorium reilianum* (Torres-Montalvo, 1998) may also fall into this category. These latter pathogens may fall anywhere between risk categories 2-4 depending on the amount of genotype flow and the frequency of outcrossing, but we hypothesize that the breeding approach based on resistance gene pyramids will be optimal.

Pathogens that exhibit mixed reproduction that includes regular recombination pose a different type of evolutionary risk that requires a different breeding strategy. These pathogens exhibit higher genotype diversity as a result of recombination and have greater potential for local adaptation to a changing environment (such as introduction of a new resistant variety). After a mutation to virulence occurs, it can be recombined into many different genetic backgrounds, and it can be recombined with other virulence mutations that occur at unlinked loci. A recombining pathogen has the potential to create a virulence allele pyramid in response to selection imposed by a resistance gene pyramid, thus pyramids are not an optimum approach for these pathogens. And when a virulent mutant migrates to a new population, the mutant virulence allele can be recombined into many new genetic backgrounds in the locally adapted recipient population (an example of gene flow). Pathogens with a mixed reproduction system and a low potential for gene flow are placed in risk category 3 in our model. For these pathogens, we believe the breeding effort should focus on quantitative resistance instead of major gene resistance. As a result of the mixed reproduction system, the mutation for virulence can be recombined into many different genetic backgrounds, and it is likely that one or more of the resulting pathogen strains will have a high level of fitness on the corresponding resistant cultivar. A major resistance gene is likely to break down quickly under these circumstances. In the presence of quantitative resistance, the pathogen may evolve increased aggressiveness (the counterpart of plant quantitative resistance), which also is likely to be a quantitative character. For example, a continuum in aggressiveness has been described in genetic crosses with *Nectria*

*haematococca* on peas (Funnell et al., 2000; Tegtmeier & VanEtten 1982) and *Ustilago hordei* (Caten et al., 1983; Pope & Wehrhahn, 1990). To evolve a higher level of aggressiveness, the pathogen must recombine a number of alleles at independent loci into a single genotype (a coadapted gene complex). Sexual reproduction will tend to break up these coadapted gene complexes in pathogen genotypes that have a high level of aggressiveness, thus the increase in aggressiveness in sexually reproducing pathogens may occur quite slowly and escape notice if asexual spores are not widely dispersed. If quantitative resistance is not available, then major resistance genes can be deployed in rotations through time or space. Rotations on a regional basis are expected to be effective against low gene flow pathogens because the virulent mutants that arise to overcome a major resistance gene in one region will not be likely to emigrate to other regions. Rotations of major resistance genes through time will produce disruptive selection that may prevent different virulence mutations from accumulating in the same genotype. Examples of pathogens that appear to fall into this category are the rice sheath blight pathogen *Rhizoctonia solani* and the soybean root rot pathogen *Phytophthora sojae*.

The highest risk pathogens are pathogens with a mixed reproduction system and a high degree of gene flow. These pathogens fall into risk category 9 in our model. We believe that these pathogens will require the greatest effort to achieve durable resistance because the mutations to virulence can be recombined into many genetic backgrounds until a pathogen clone with a high fitness appears, and then this adapted genotype can be dispersed across long distances and into new populations. Once in the new population, the pre-adapted clone can increase in frequency by asexual reproduction or the virulence mutation can be recombined into new, locally adapted genotypes in the recipient population. For pathogens in this risk category, we suggest the breeding effort should concentrate on quantitative resistance that will need to be renewed regularly to stay ahead of the pathogen. If quantitative resistance is not available, then major resistance genes should be managed aggressively, including development of cultivar mixtures and multilines that can be used in combination with regional and temporal deployment strategies. Examples of pathogens that fall into this category are *Blumeria graminis* f. sp. *hordei*, causing barley powdery mildew, and the new sexual populations of *Phytophthora infestans*. The evolutionary potential of *P. infestans* populations may still be somewhat limited due to the limited number of founder individuals that exist outside of Mexico, but our model predicts that the introduction of sexual reproduction into these populations will increase their evolutionary potential considerably. However, it remains to be seen whether the new sexual populations can break down resistance (especially quantitative resistance) faster than the old asexual populations.

## Concluding comments

We have cautiously entered the era of genetic engineering of our major crops. While it is clear that genetic engineering technologies offer great potential, the initial enthusiasm was dampened after public acceptance of GMO products was affected by the perceived risk inherent in a new technology. Acceptance also was diminished because GMOs present a number of uncharacterized risks to agroecosystems (hybridization with weedy species, effects on non-target organisms, horizontal gene transfer) that require further investigation. Another, though less publicized, risk is that genetically engineered resistance genes will face the same fate (boom and bust cycles) as the major resistance genes that were moved into our basic food crops by traditional breeding methods over the last hundred years. As cloning and characterization of resistance genes proceeds, it will become progressively easier to manipulate genetic resistance and transfer resistance genes within species (really a form of gene therapy) and among species. Our present knowledge indicates that plants evolved LRR-types of receptors to recognize a diverse array of pathogen elicitors, and it is likely that pathogens coevolved with these receptors for millions of generations before agriculture arose. With this long history of coevolution, it seems unlikely that we will be able to eliminate plant diseases simply by engineering new receptors and putting them into our existing crops. Pathogens will continue to evolve. But genetic engineering offers new potential to stay a few steps ahead of the pathogen. Genetic engineering can be used to recombine receptors quickly, creating novel pyramids of major resistance alleles (receptors) that can be transferred into plants as a cassette of linked genes. Thus it may become possible to create a resistance gene pyramid more quickly through a single transformation step rather than through a series of hybridizations and backcrosses. Of course, plants already have evolved cassettes of linked resistance genes over evolutionary time scales, and pathogens

are still with us. As explained earlier, resistance gene pyramids lose their effectiveness quickly when faced with a recombining pathogen population. Genetic engineering also will make it possible to synthesize multilines quickly and efficiently by inserting different single resistance alleles into agronomically superior crop genotypes. This approach may allow us to better mimic the distribution of resistance that occurs in natural ecosystems, but it is unlikely to eliminate the pathogen. It is most likely that pathogen populations will continue to evolve and respond to the new forms of genetic resistance that we deploy through genetic engineering. But with careful management of these new, engineered resistance genes, we may be able to create truly durable forms of genetic resistance. The best way to insure the durability of these new resistance genes is to manage them wisely using knowledge of the evolutionary potential of the pathogen population.

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