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Multiline Varieties and Disease Control
I. The "Dirty Crop" Approach with Each Component Carrying a Unique Single Resistance Gene

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Summary. The effects of the widespread use of "dirty crop" or "partially resistant" multilines on the racial composition of a pathogen population were investigated using simple theoretical models. It was found that the evolutionary changes in the pathogen attacking multiline varieties depend critically on two factors - the level of selection against unnecessary genes for virulence(s) and the number of lines in the multiline (n):

(i) If $s > 0.5$, then multilines will stabilize the racial composition of the pathogen population and simple races, carrying a single gene for virulence, will be the predominant biotypes.

(ii) If $s < 1/2\,(n-1)$ when unnecessary genes for virulence are additive in their effects in reducing pathogen fitness, or $s < 1/n$ when unnecessary virulence genes act multiplicatively to reduce pathogen fitness, then the use of a multiline will lead to the development of a superrace which can simultaneously attack all the component lines.

(iii) If $1/2 > s > 1/2\,(n-1)$ for the additive model, or $1/2 > s > 1/n$ for the multiplicative model, the use of multiline varieties will stabilize the pathogen population, but with complex races, carrying two or more virulence genes, predominant.

These findings are discussed in relation to the potential of multiline varieties as a means of achieving stable, long-term control of plant diseases. It is concluded that "dirty crop" and "partially resistant" multilines will provide stable disease control in crop plants only in limited and relatively rare circumstances.

Key words: Multiline Varieties - Disease Control - Stabilizing Selection

Introduction

The use of multiline varieties in self-pollinating crops has been advocated repeatedly in the literature since the late nineteenth century, in part, because of their potential in disease control (Simmonds 1962; Browning and Frey 1969). However only three groups are actively developing multiline varieties and their programs are based on two radically different philosophies for the achievement of disease control (Jensen 1952; Borlaug 1958; Browning and Frey 1969).

In one approach, designated the "clean crop" approach (Marshall 1977), all component lines of the mixture would be resistant to all prevalent races of the disease(s) to be controlled (Jensen 1952; Borlaug 1958). The aim of this scheme is to keep the crop as free of disease as possible, and at the same time, to reduce the threat of catastrophic disease losses following shifts in the racial composition of the pathogen population.

In the second approach, designated the "dirty crop" approach (Marshall 1977), each line in the mixture also carries a different single gene resistance; however, none of the lines are resistant to all known races of the pathogen. Therefore, such multilines will be regularly attacked by the pathogen. Frey et al. (1973, 1975) have argued that such multilines should protect the crop in two ways:

(i) They should stabilize the race structure of the pathogen population. This argument is based on the premise that stabilizing selection against races carrying multiple genes for virulence (van der Plank 1963, 1968) will ensure that "simple" races, carrying a single virulence gene, dominate the pathogen population.

(ii) Since each component of the multiline would be attacked by only one race of the stabilized pathogen population, the remaining lines would act as spore traps reducing the rate of spread of the disease. In this way, multiline cultivars would have an effect similar to polygenic nonspecific or horizontal (van der Plank 1963) resistance in delaying the intracrop build-up of the pathogen.

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The "dirty crop" approach using partially resistant multilines, if it should prove valid, has a significant potential advantage over the "clean crop" approach using completely resistant multilines. It would indefinitely extend the useful life of "strong" resistance genes (van der Plank 1963) including those that have broken down in the past. Hence, it would free the breeder from the onerous task of continually isolating and evaluating new sources of resistance.

However, the validity of the proposal put forward by Frey and his colleagues rests heavily on the assumption that simple races will dominate the pathogen population and this assumption is open to question on two grounds. These are, first, whether van der Plank's (1963) axiom that complex pathogen biotypes are less fit than simple biotypes on susceptible host genotypes is generally valid, and further, whether this loss of fitness can be directly attributed to the virulence genes per se. Second, given that stabilizing selection is a general, if not universal phenomenon, whether it is strong enough to prevent the development of complex races on multilines; that is, whether stabilizing selection against races carrying additional virulence genes will outweigh the selection pressure towards greater virulence which arises from the fact that more complex races can grow on more lines and, hence, spread more rapidly through the host population.

In this paper we attempt to (i) define, using the simple models first developed by Groth (1976) and Groth and Person (1977), the level of stabilizing selection required to prevent the development of complex pathogen races on multiline varieties, and (ii) assess from published experimental results how often the required levels of selection are likely to be met in practice. Obviously, if it can be demonstrated that stabilizing selection, where it exists, is too weak to prevent the development of a superrace on a multiline, then the questions of its generality and ultimate cause become academic.

The Models

Additive Selection Models

Two line mixtures. Consider an equiproportional mixture of two diploid host genotypes which are identical except for the fact that each is homozygous for a different dominant resistance to a specified haploid pathogen. The resistance genes in the host may be allelic or nonallelic, and for simplicity we will assume that each resistance gene recognizes a single unique avirulence gene in the pathogen, i.e., the host-pathogen system under discussion conforms to the gene-for-gene concept originally developed by Flor (1956).

Table 1. Relative fitnesses of pathogen biotypes on a two-line multiline assuming an additive fitness effects model

Class	Pathogen Biotype Description	Genotype	Host Genotype R_1R_1	Host Genotype R_2R_2	Mean Fitness on Mixture
1	Single effective gene for virulence	$a_1A_2A_3$	1	0	0.5
		$A_1a_2A_3$	0	1	0.5
2	Single effective gene and single unnecessary gene for virulence	$a_1A_2a_3$	1-s	0	(1-s)/2
		$A_1a_2a_3$	0	1-s	(1-s)/2
3	Two effective genes for virulence	$a_1a_2A_3$	1-s	1-s	1-s
4	Two effective genes and one unnecessary gene for virulence	$a_1a_2a_3$	1-2s	1-2s	1-2s

Each of the components in the mixture can and will eventually, be attacked by four classes of pathogen biotypes, viz., those that carry one effective virulence gene, with and without additional unnecessary virulence genes, and those that carry both effective virulence genes, with and without unnecessary virulence genes. The assumed relative fitness of each of these four classes of pathogen biotypes on each host genotype, and their mean relative fitness as over the population as a whole, are shown in Table 1. Basically we assume that (i) pathogen biotypes carrying only necessary genes for virulence have equal fitness, (ii) each unnecessary gene for virulence reduces their fitness by a constant amount, s, and (iii) two or more unnecessary genes for virulence are additive in their effects in reducing the fitness of the pathogen.

The assumption of annual reconstitution of the multiline ensures that its composition is stable with time. We also assume that the crop is grown over a large area so that it is the major factor influencing the evolution of virulence in the local pathogen population. Under these assumptions, the mean fitnesses of the parthogen biotypes on the mixture in Table 1 can be used directly to predict the composition of the pathogen population. The biotype, or class of biotypes, with highest mean fitness will in time dominate the pathogen population; the remainder will be absent or at low frequency in the population. Under this model, lines carrying unnecessary genes for virulence are less fit than lines carrying only effective genes for virulence. For example, the relative fitnesses of class 1 and 2 biotypes are 0.5 and $(1 - s)/2$, respectively, while those for class 3 and 4 biotypes $1 - s$ and $1 - 2s$, respectively. Therefore, class 2 and 4 biotypes will be selectively lost from the population, provided $s > 0$, that is, provided there is stabilizing selection against unnecessary virulence genes.

Therefore, either class 1, the simple races carrying one effective virulence gene, and/or class 3, the "superrace" carrying both effective genes, will dominate the pathogen population. If the mean fitness of class 3 biotypes is greater than that of class 1, that is,

$$(1 - s) > 1/2$$

or

$$s < 0.5$$

then a superrace will develop. Alternatively, if $s > 0.5$, the pathogen population will consist principally of simple races. Finally, if $s = 0.5$, both class 1 and class 3 have an equal chance of survival. In the latter case, the pathogen population will be polymorphic, and the relative proportions of each class of biotypes will vary stochastically.

n-line mixtures. Consider the same model as before for an n-line mixture. Here, 2n classes of pathogen biotypes may parasitize the host population - those with one, two, ..., k, ..., n effective virulence genes with and without one or more unnecessary genes for virulence. The relative fitnesses of the pathogen biotype are given in Table 2.

As before, biotypes carrying unnecessary genes for virulence are invariably less fit than those with only effective genes for virulence and will be eliminated from the population. Simple races carrying a single effective gene for virulence will dominate the pathogen population, if

$$1/n > 2(1 - s)/n$$

or

$$s > 1/2 .$$

Lines carrying two genes for virulence will dominate the pathogen population if

$$1/n < 2(1 - s)/n > 3(1 - 2s)/n$$

or

$$1/2 > s > 1/4 .$$

Complex pathogen races carrying k effective genes for virulence will dominate the pathogen population if

$$(k-1)[1-(k-2)s]/n < k[1-(k-1)s]/n > > (k+1)(1-ks)/n$$

or

$$1/2(k-1) > s > 1/2k \qquad (k \leqslant n-1) .$$

Finally, a superrace which can attack all component lines of the mixture will develop if

$$n[1-(n-1)s]/n > (n-1)[1-(n-2)s]/n$$

or

$$s < 1/2(n-1).$$

Table 2. Relative fitnesses of pathogen biotypes on an n-line multiline assuming an additive fitness effects model

Class	Pathogen Biotype Description	Genotype	Host Genotype R_1R_1	Host Genotype R_2R_2	Host Genotype R_nR_n	Mean Fitness on Mixture
1	Single effective gene for virulence	a_1--A_k--$A_nA_{(n+1)}$	1	0	0	1/n
		A_1--A_k--$A_nA_{(n+1)}$	0	1	0	1/n
		A_1--A_k--$a_nA_{(n+1)}$	0	0	1	1/n
2	Single effective gene and single unnecessary gene for virulence	a_1--A_k--$A_na_{(n+1)}$	(1-s)	0	0	(1-s)/n
		A_1--a_k--$A_na_{(n+1)}$	0	(1-s)	0	(1-s)/n
		A_1--A_k--$a_na_{(n+1)}$	0	0	(1-s)	(1-s)/n
2k-1	k effective genes for virulence	a_1--a_k--$A_nA_{(n+1)}$	[1-(k-1)s]	[1-(k-1)s]	0	k[1-(k-1)s]/n
2k	k effective genes and a single unnecessary gene for virulence	a_1--$a_kA_na_{(n+1)}$	(1-ks)	(1-ks)	0	k(1-ks)/n
2n-1	n effective genes for virulence	a_1--a_k--$a_nA_{(n+1)}$	[1-(n-1)s]	[1-(n-1)s]	[1-(n-1)s]	[1-(n-1)s]
2n	n effective genes and a single unnecessary gene for virulence	a_1--a_k--$a_na_{(n+1)}$	(1-ns)	(1-ns)	(1-ns)	(1-ns)

As before, if $s = 1/2k$ the pathogen population will be polymorphic for races carrying (k - 1) and k genes for virulence, with the relative proportions of the various biotypes varying stochastically.

Multiplicative Selection Models

n-line Mixtures. For this model the relative fitnesses of the 2n classes of pathogen biotypes which can attack an n-line mixture are given in Table 3. Again, we see that biotypes carrying unnecessary genes for virulence are invariably less fit than those with only effective virulence genes and will be eliminated from the population. We also see simple races carrying a single effective virulence gene will dominate the pathogen population only if,

$$1/n > 2(1 - s)/n$$

or

$$s > 1/2$$

as in the case of the additive fitness model. Lines carrying two genes for virulence will dominate the pathogen population if

$$1/n < 2(1 - s)/n > 3(1 - s)^2/n$$

or

$$1/2 > s > 1/3 .$$

Complex pathogen races carrying k effective genes for virulence will be the predominant biotypes if

$$(k - 1)(1 - s)^{(k-2)}/n < k(1 - s)^{(k-1)}/n > > (k + 1)(1 - s)^k/n$$

or

$$1/k > s > 1/(k + 1) .$$

Table 3. Relative fitnesses of pathogen biotypes on an n-line multiline under a multiplicative fitness effects model

Class	Pathogen Biotype Description	Genotype	Host Genotype R_1R_1	Host Genotype R_kR_k	Host Genotype R_nR_n	Mean Fitness on Mixture
1	Single effective gene for virulence	$a_1 - A_k - A_n A_{(n+1)}$	1	0	0	1/n
		$A_1 - a_k - A_n A_{(n+1)}$	0	1	0	1/n
		$A_1 - A_k - a_n A_{(n+1)}$	0	0	1	1/n
2	Single effective gene and single unnecessary gene for virulence	$a_1 - A_k - A_n a_{(n+1)}$	1-s	0	0	(1-s)/n
		$A_1 - a_k - A_n a_{(n+1)}$	0	1-s	0	(1-s)/n
		$A_1 - A_k - a_n a_{(n+1)}$	0	0	1-s	(1-s)/n
2k-1	k effective genes for virulence	$a_1 - a_k - A_n A_{(n+1)}$	$(1-s)^{k-1}$	$(1-s)^{k-1}$	0	$k(1-s)^{k-1}/n$
2k	k effective genes and a single unnecessary gene for virulence	$a_1 - a_k - A_n a_{(n+1)}$	$(1-s)^{k}$	$(1-s)^{k}$	0	$k(1-s)^{k}/n$
2n-1	n effective genes for virulence	$a_1 - a_k - a_n A_{(n+1)}$	$(1-s)^{n-1}$	$(1-s)^{n-1}$	$(1-s)^{n-1}$	$(1-s)^{n-1}$
2n	n effective genes and a single unnecessary gene for virulence	$a_1 - a_k - a_n a_{(n+1)}$	$(1-s)^{n}$	$(1-s)^{n}$	$(1-s)^{n}$	$(1-s)^{n}$

Finally, a superrace which is virulent on all lines in the mixture will develop if

$$(1 - s) > (n - 1)(1 - s)^{(n-2)}/n$$

or

$$s < 1/n .$$

Implications of the Model

The results indicate that the use of "partially resistant" or "dirty crop" multilines may:

(i) Stabilize the racial composition of the pathogen population with simple races, carrying a single gene for virulence, as the predominant biotypes (Browning and Frey 1969), or

(ii) Stabilize the racial composition of the pathogen population but with complex races, carrying a number of virulence genes, predominating (Marshall 1977), or

(iii) Lead to the development of a "superrace" which can attack all components of the mixture (Caldwell 1966).

Thus, they confirm, in at least some circumstances, all the previous intuitive predictions concerning the evolutionary behavior of pathogens on partially resistant multilines. The outcome in any particular circumstance (crop and pathogen) depends critically on two factors viz. the level of selection against unnecessary genes for virulence, s, and the number of lines in the multiline, n. Groth (1976) and Groth and Person (1977) reached the same conclusions using different procedures.

Simple races will dominate the pathogen population only if $s > 1/2$. This conclusion holds regardless of the fitness model involved or the number of lines in the multiline, and is therefore a "robust" prediction in the sense of Levins' (1968). A "superrace" will develop on the multiline only if $s < 1/2(n-1)$, when the individual virulence genes in the pathogen are additive in their effects in reducing fitness, and $s < 1/n$ when

the virulence genes are multiplicative in their effects on the fitness of the pathogen. Therefore, a superrace is less likely to develop on a multiline if unnecessary genes for virulence are additive rather than multiplicative in reducing pathogen fitness. If $1/(2n - 1) < s < 1/2$ for the additive model, or $1/n < s < 1/2$ for the multiplicative model, then multilines will stabilize the racial composition of the pathogen population, but with complex biotypes predominant.

The Cost of Virulence

Obviously, if we are to make accurate predictions of the influence of the use of multiline varieties on pathogen evolution, we need accurate estimates of s, the cost of unnecessary virulence genes. Unfortunately, such estimates are generally not available. Indeed, the whole concept of a cost of unnecessary virulence genes, and hence "stabilizing selection" as defined by van der Plank (1968, 1975), remains controversial (Browning and Frey 1969; Watson 1970; Pryor 1977).

Numerous studies of competition between individual isolates of different pathogenic races have been reported in the literature. However, as several authors (van der Plank 1975; Leonard 1977; Pryor 1977) have stressed, such studies are seldom of any value in estimating the intensity of stabilizing selection, because the effects of genes for virulence are confounded with the effects of other genetic differences among the isolates. Using the best available data, Leonard (1977) estimated that $0.12 < s < 0.42$ for several different pathogens.

Taken at face value, Leonard's (1977) estimates of the cost of unnecessary virulence genes argue that the level of stabilizing selection will seldom be of sufficient intensity ($s > 0.5$) to ensure the stabilization of the pathogen population with simple races, carrying a single gene for resistance as the predominant biotypes. However, in theory, it would still be possible, by manipulation of the number of lines in the mixture to (i) prevent the development of a superrace and (ii) to stabilize the racial composition of the pathogen population with biotypes of intermediate complexity predominant and have the multiline provide adequate protection against disease loss. The critical questions in this case is how large should the number of lines n, be to achieve these objectives.

Number of Lines Required in a Multiline

(i) To prevent the development of a superrace

It is shown earlier that a superrace will develop on a multiline only if $s > 1/2(n - 1)$ for the additive model, and $s > 1/n$ for the multiplicative model. We can calculate from these expressions the minimum number of lines required in a multiline in terms of the level of stabilizing selection against unnecessary virulence genes, to prevent the development of a pathogen "superrace." These are, $n > (1 + 2s)/2s$ for the additive model, and $n > 1/s$ for the multiplicative model, respectively. The values of n which satisfy these relationships for each model, calculated in the light of Leonard's (1977) estimates of s indicate that at least 9 components would be required in a multiline for a breeder to be reasonably sure that a pathogen superrace would not develop.

Table 4. Number of lines required in a multiline to prevent the development of a pathogen superrace

Model	Level of Stabilizing Selection (s)	
	0.12	0.42
Additive	6	3
Multiplicative	9	3

(ii) To stabilize pathogen population with adequate disease control

A critical question here is - what proportion of a mixed host population can be susceptible to each pathogen biotype, and the population still escape significant disease damage? Estimates of the allowable proportion of susceptibles in a multiline range from 6 to 40 percent (Browning and Frey 1969). The acceptable proportion of susceptibles will vary with the crop and disease in question, as well as with environmental conditions. For the purposes of the present discussion, we will consider a range of values 5 to

Table 5. Number of lines required in a multiline to provide adequate disease protection for varying levels of stabilizing selection (s) and acceptable proportion of susceptible plants (p) in the host population

Level of Stabilizing Selection	No. Virulence Genes in Pathogen	Acceptable Proportion of Susceptible Host Plants (p)			
(s)	(k)	0.05	0.10	0.20	0.40
Additive model					
0.12	5	100	50	25	13
0.42	2	40	20	10	5
Multiplicative model					
0.12	8	160	80	40	20
0.42	2	40	20	10	5

40 percent encompassing all the estimates reported in the literature.

Once we have defined the acceptable proportion of susceptibles in a multiline, we can calculate for a given level of stabilizing selection (which defines the number of virulence genes carried by each pathogen biotype), the number of lines required in a multiline to provide adequate disease protection (Table 5). This number turns out to be relatively large. For most crops and diseases the required number of single resistance genes is unlikely to be available to breeders at any one time. Multilines will only be feasible in practice where is strong stabilizing selection against unnecessary virulence genes in the pathogen ($s \geqslant 0.42$) and where the proportion of host plants susceptible to each pathogen biotype is large ($p = 20$-40 percent). Hence, "partially resistant" or "dirty crop" multilines appear to offer an effective solution to the problem of achieving more permanent forms of disease control in crop plants in relatively limited circumstances.

Deficiencies of the Models

As pointed out by Levins (1968), there is no single, best all-purpose model of a particular evolutionary process. In particular, it is not possible to maximize simultaneously in a model generality, realism and precision. The models used here sacrifice generality and realism for precision. Thus we can define very precisely the level of stabilizing selection required to prevent the development of a superrace on a multiline under the assumptions of our models. However, many of these assumptions are biologically unrealistic.

We have assumed that the levels of stabilizing selection against unnecessary virulence genes is (i) the same for all such genes, (ii) constant over time (generations of the pathogen) regardless of climatic conditions, and (iii) invariant with changes in the genetic background of the virulence genes in the pathogen and resistance genes in the host. All of these assumptions are likely to be invalid in practice. Indeed, substantial evidence already exists to indicate that the level of stabilizing selection varies from locus to locus, with environmental conditions and with the background genotype of both the host and pathogen (van der Plank 1963, 1968, 1975; Leonard 1977).

Future studies will be concerned with the effects of removing these assumptions and the development of more realistic models of pathogen evolution on mixed host populations.

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