Martin S. Wolfe
Plant Breeding Institute, Cambridge, England

John A. Barrett
University of Cambridge, England

Can We Lead the Pathogen

Cereal growing in the United Kingdom is intensive and is concentrated mainly on winter and spring crops of wheat and barley. Fertile soils, good rainfall, and a mild climate ensure both high-yielding crops and a plentiful supply of major pathogens. Constraints against these pathogens include disease-resistant cultivars and fungicides.

Breeding for resistance to some pathogens has proved to be relatively simple. For example, moderate resistance to eyespot (*Pseudocercosporella herpotrichoides*) from the once widely grown winter wheat Cappelle-Desprez has been transferred to the majority of British wheats and has protected the crop for more than 20 yr over a million or so hectares per year. There have also been some notable successes with fungicidal control of potentially major diseases. Organomercurial control of bunt (*Tilletia caries*) and other seedborne organisms is a long-established practice, and in recent years, carboxin has provided inexpensive and comprehensive control of loose smut (*Ustilago nuda*).

The foliar diseases, principally powdery mildew of barley (*Erysiphe graminis* f. sp. *hordei*) and yellow rust of wheat (*Puccinia striiformis*), present more of a problem. Yellow rust of wheat is potentially most damaging and can lead to almost total crop loss. That the disease has caused no major national problem for many years is a tribute to the vigilance and efforts of breeders, pathologists, advisors, fungicide specialists, and, of course, farmers. This effort is costly, however, both directly, through breeding, provision and application of fungicides, advisory work, and testing, and indirectly, through limitations placed on breeding programs by the need for resistance and loss of yield from cultivars lacking adequate resistance.

An attempt to stabilize the position and reduce costs is being made by exploiting what has been termed inherent "durable resistance" by Roy Johnson at the Plant Breeding Institute. Because the likely longevity of any introduced resistant cultivar is impossible to predict, starting a breeding program with parents whose resistance has endured for many years and over a large area is logical. Johnson and other colleagues are now engaged in transferring a chromosome that controls most of the phenotypic characteristics of some durably resistant wheat varieties into new, higher-yielding cultivars (10).

The problem with barley mildew is more difficult. Minor differences in durability have been observed among cultivars, but none has persisted for more than 3 or 4 yr without obvious loss of resistance. Consequently, the disease has been a chronic problem in barley production in the United Kingdom, with an annual loss of about $80 million. Because no relation was apparent between the kinds of resistance used and their durability, some radical change in the way of using resistant cultivars was considered.

Our collaboration began in 1972 with the appraisal of various strategies for deploying and using resistant cultivars. The most suitable system appeared to be mixing cultivars of different resistances, an idea expressed in a general way by Jensen (9) and developed as a multiline strategy by Borlaug (4) and Browning and Frey (5). The starting material was a range of cultivars susceptible to the disease. Our information from race surveys showed that the cultivars could be classified into different resistance phenotypes and that the pathogen population selected on each was usually limited to it (13), ie, relatively few pathogen phenotypes could attack more than one resistance phenotype. Our task was to coordinate pathologic analyses in field and laboratory with genetic and mathematical studies to understand the operation of the system and to develop its application.

The Theory of Pathogen Behavior in Mixtures

In a mixture strategy, strains of the host plant that differ in resistance characters are grown together. The components may show a phenotype-for-phenotype interaction with the pathogen (1,2) or may differ only in degree of resistance (8). In essence, the principles underlying disease control in mixtures are the same in both types. If the components are completely resistant to the prevailing pathogen population, no disease will develop. If one of the components is susceptible, some of the propagules liberated from the infected plants will be distributed over adjacent plants. Inevitably, some propagules will reinfect the plant on which they were produced, causing disease buildup. Propagules that land on other plants, however, will not necessarily be able to develop as well as on the original infected plant, especially if a classic gene-for-gene interaction occurs between host and pathogen, where the pathogen cannot attack unless it has the corresponding virulence gene. In mixtures in which each component is susceptible to a different part of the pathogen population or in which the components differ in degree of resistance and are susceptible to all of the pathogen population, the propagules released from their "parent" plants will be able to infect only some of the plants in the mixed stand. Thus, there will be a net decrease in disease incidence and severity.

If there is a phenotype-for-phenotype interaction that can also generate pathogen genotypes capable of attacking more than one component, the possibility of the development of more complex races

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Astray?

or even the so-called super-race must be considered. In some fungal diseases, multiple virulent pathogen genotypes are at a disadvantage to single virulent pathogen genotypes on the compatible host. Van der Plank maintains (12) there is always selection against virulence genes that are unnecessary for infection of a host phenotype. Thus, in a mixture of host plants, the pathogen has an evolutionary dilemma to solve: 1) For maximum success on any one component, the pathogen must possess the minimum number of virulence genes, but then it is unable to attack all components of the mixture. 2) A genotype able to attack more than one component carries the penalty of reduced efficiency of infection on any one component.

Which solution is favored by natural selection depends on a number of factors, but the principal forces are: (i) the relative disadvantage of virulence genes not required for infection, (ii) the ability of pathogen genotypes without the corresponding virulence to grow on a given host, and (iii) the rate of propagule exchange among plants in the stand.

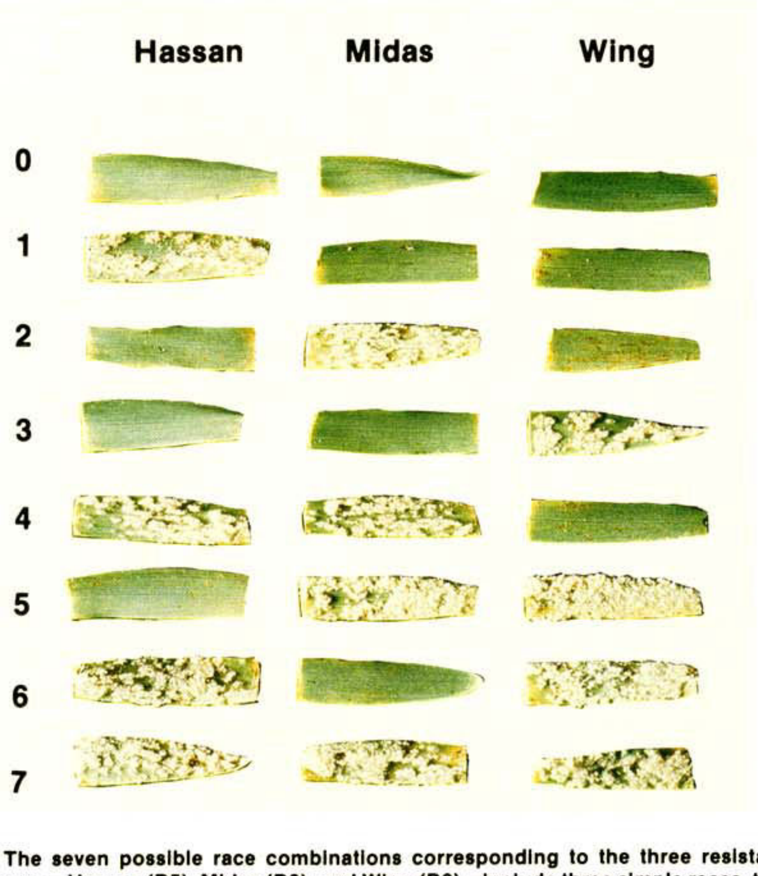


Fig. 1. The seven possible race combinations corresponding to the three resistance phenotypes—Hassan (R5), Midas (R3), and Wing (R6)—include three simple races, three intermediate races, and one complex race.

The pathogen genotypes able to grow on more than one component have a higher mean fitness at high rates of propagule exchange than at low rates. Thus, at high rates of exchange, flexibility of the pathogen to attack more than one component is an advantage. Conversely, the mean fitness of pathogen genotypes able to attack one component well and the remainder not so well declines as the rate of propagule exchange increases. This does not mean that complex races will always supplant simple races if the rate of propagule exchange is high, because the relative fitness of the more complex genotypes over the simple races depends on the magnitude of the selection coefficients in (i) and (ii); only the direction in which the fitness will change is indicated.

At the start of the disease season, genotypes able to attack more than one component tend to be more successful than simpler genotypes because they can attack a larger proportion of the components. The relative frequency of the more complex pathogen genotypes, therefore, tends to rise initially. If the rate of propagule exchange is low, however, the superior reproductive ability of the simpler pathogen genotypes on each component can lead to an increase in the relative frequency of the simpler genotypes in succeeding generations. Thus, at the end of the season, the simpler pathogen genotypes can show a net increase in relative frequency.

The evolutionary dynamics of a pathogen in a mixture can lead to increases and decreases in the frequencies of the different pathogen genotypes during the disease season, and there will not necessarily be a constant increase or decrease in any one genotype. Whether a

Table 1. Percentage of mildew infection on the upper three leaves of four mixtures of three cultivars each compared with the mean infection on the same three cultivars grown separately

Components	Mildew infection[a] Observed	Expected
Athos-Georgie-Mazurka	6.5	20.3
Hassan-Georgie-Wing	9.3	19.4
Athos-Ambre-Mazurka	2.8	9.7
Athos-Sundance-Mazurka	1.7	7.9
Mean	5.1 36%	14.3

[a]Data from the ADAS/PBI collaborative trials in 1978.

Table 2. Mean time course of mildew epidemic development in four barley cultivar mixtures relative to the means of the components grown separately

Days from sowing	Disease level relative to mean of pure stands
37[a]	100[a]
53	85
58	67
65	38
73	40
80	46
86	58
95	62
100	58

[a]Estimated.

mixed host stand selects simple, intermediate, or complex races is not easily predictable (1–3,7). Because of the trade-off between efficient infection of one component with low infection of the remaining components, as well as the flexibility to attack more than one component with reduced infection efficiency, disease levels will be reduced compared with the mean of the components grown as pure stands.

Because a flexible response by the pathogen is obviously an advantage, modifier genes that can improve the fitness of the more complex genotypes are favored. This process would be slower than selection of the virulence genes themselves but could lead to complex genotypes evolving in systems where simple genotypes had been favored, thus reducing effectiveness of the mixture for disease control. An essential part of this approach to disease control is that no one mixture be used for an extended period of time; this reduces the possibility of fitness modifiers of the complex pathogen genotypes being selected. The mixture strategy is a dynamic control system in which the changes *must* be rung to prevent adaptation of the pathogen to the control measures.

Disease Control

Consistent with the prediction from the model, simple mixtures of three cultivars that were susceptible in the field to different races produced dramatic reductions in amounts of mildew (Table 1). The data are a sample of observations from trials done nationally in collaboration with ADAS (Agricultural Development and Advisory Service) during 1978. Similar observations have been made in many other trials during the past 4 yr; eg, one seed company observed the Athos-Georgie-Mazurka mixture at more than 60 farms and found that the average disease prevalences were almost identical with those in Table 1.

Relative epidemic reduction is not constant with time. At the beginning of the season when mixtures and their corresponding pure stands are subject to infection from external sources only, the mixture has little advantage. As the season progresses and epidemic increase becomes more dependent on buildup within the crop, disease spread is slower in mixtures than in pure stands (Table 2). In the later stages of the season, epidemic progress in the mixture appears to accelerate. This is largely because infection reaches a saturation level and stops developing in pure stands but is still increasing in the relatively healthier mixtures.

In the field, epidemic reduction is effective whatever the structure of the starting population (Table 3). Pathogen virulence for the R6 cultivar dominated at Rosemaund, whereas that for R5 dominated at Worcester; in the Cambridge trial, the initial population was more complex, with relatively high levels of virulence for each cultivar. In all three examples, epidemic reduction in the mixture was considerable; the lesser effect at Cambridge was largely due to stray infection of the smaller plots.

Powdery mildew was the prevalent disease, but in some trials yellow rust (*P. striiformis*), brown rust (*P. hordei*), and *Rhynchosporium* were also observed. For all three of the latter diseases, levels were often reduced significantly in the mixture relative to the mean of the components.

Pathogen Population Dynamics in the Field

Determination of population changes in the field is technically difficult, requiring large numbers of quantitative measurements of many different subpopulations whose structures are changing rapidly. Nevertheless, we obtained some indications of trends in different trials.

Most investigations involved mixtures of Hassan (R5 resistance), Midas (R3), and Wing (R6) in equal amounts. There are seven possible race combinations corresponding to these three resistance phenotypes (Fig. 1), including three simple races, three intermediate races, and one complex race. Because pathogen populations on pure stands of these cultivars are dominated by the three corresponding simple races, we chose to investigate this host combination. In the pathogen populations on the mixtures, relative frequency of the intermediate and complex races often increases, but because the overall population size is depressed, the absolute frequency is also depressed compared with that of the races on pure stands of the component cultivars. The precise direction of selection, even between replicates of the same trial, is highly variable and unpredictable, presumably because of the effects of small environmental perturbations, as forecasted by the model.

Also consistent with the model, and contrary to expectation, was the outcome of observations made on the relative frequency of race 4 in a simple 1:1 mixture of the cultivars Hassan and Midas. In the early part of the season, race 4 was more common than race 1 or race 2 because of its initial advantage in being able to grow on both host components. As the epidemic progressed, however, its frequency declined relative to that of the two simple races. The explanation is that race 4 reproduced less well on Hassan than did race 1 and less well on Midas than did race 2. Consequently, over several generations, the two simple races eventually predominated, ie, the mean fitness of each of the simple races in the population was greater than that of the complex race.

That each race is a population and is therefore variable became clear in observations made by Chin (6), working with us at Cambridge. He compared samples of race 6 from pure stands of Hassan and Wing with samples from Hassan and Wing growing in a mixture of Hassan, Midas, and Wing. Isolates of race 6 from pure stands of Hassan grew better on test seedlings of Hassan than of Wing; the reverse was true for isolates from pure stands of Wing. Isolates of race 6 from the mixture did not show this differentiation, growth on Hassan and Wing being equal. Overall, their performance on the two test cultivars appeared to be poorer than that of race 6 samples from pure stands, indicating adaptation due to selection of modifier genes.

We cannot predict how a pathogen will respond to large-scale use of a single mixture such as Hassan-Midas-Wing. Indications so far, however, are that disease control lost in a mixture is more likely to be a gradual process of erosion than a sudden breakdown. The main pointers are first, there is no rapid, widespread, unidirectional selection of the complex race. Indeed, such selection has not occurred even on the small area of one farm where most of our experiments were done during the last 4 yr in an attempt to produce the worst possible result by maximizing survival of the more complex races. Second, variability exists within complex races so that selection for individuals specialized to each of the host components can still occur, providing a residual mixture effect. Third, the individual host cultivars have different degrees of susceptibility to the most virulent corresponding simple races. So, even if a race did occur that combined the virulence of the most virulent simple races, the mean infection level would tend to be less than the mean infection of the components grown separately.

However, we do not wish to see selection pressure applied to the extent that such responses are invoked. The overall strategy calls for use of different mixtures to exert a rapidly changing disruptive selection on the pathogen population. Such a system is consistent

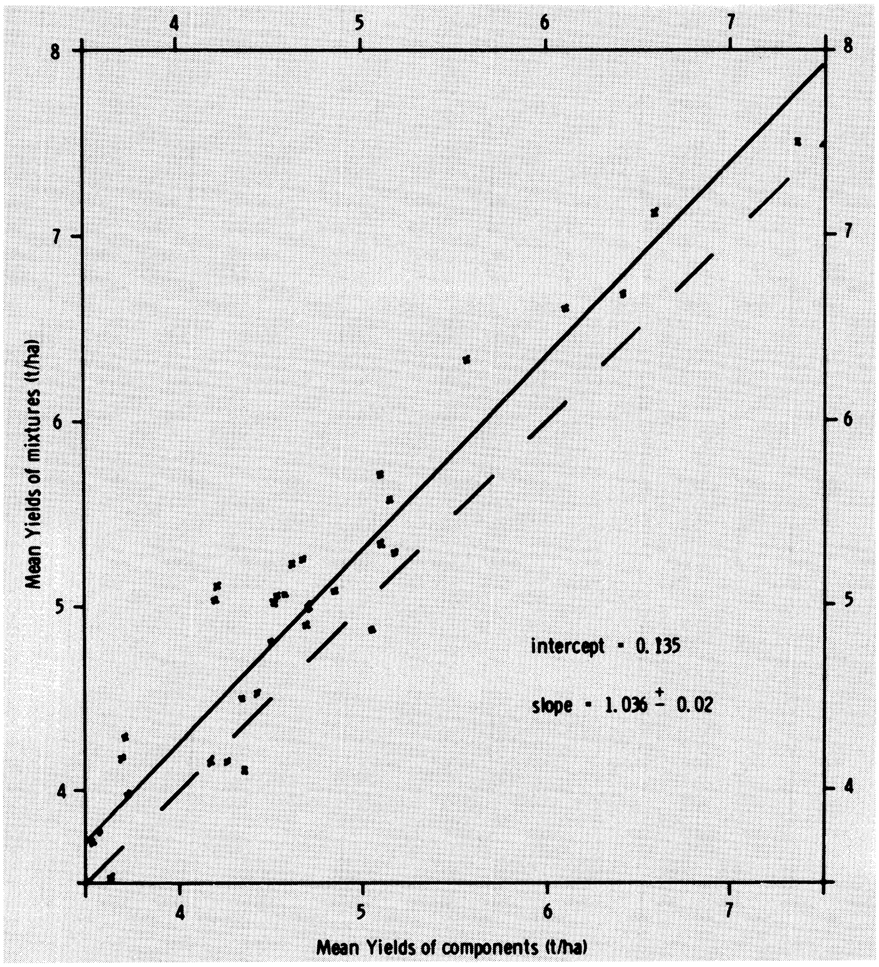


Fig. 2. Regression of the yields of 32 mixtures (each containing at least one higher-yielding cultivar) on the means of their components grown alone. The mean yield increase of the mixtures over their components' mean was 6.5%.

Table 3. Mildew disease reduction in barley mixtures infected with different pathogen populations

Cultivar	Rosemaund[a]	Worcester[a]	Cambridge[b]
R4 pure plot	8	2	31
R5 pure plot	4	29	13
R6 pure plot	21	2	21
Mean	11	11	22
R4-R5-R6 mixture plot	4	3	13
Percentage	36	27	59

[a]Large plots (2 ha).
[b]Small plots (3 m^2).

Table 4. Mean yields of six leading spring barley cultivars and of mixtures to which they contributed, compared with mean yields of mixture components grown as pure stands[a]

Cultivar and number of sites	Mean yield of cultivar (t/ha)	Mean yield of mixture components grown alone (t/ha)	Mean yield of mixtures containing cultivar (t/ha)
Sundance (7)	4.99	4.78	5.05
Georgie (10)	4.88	4.65	5.10
Athos (9)	4.74	4.70	5.09
Mazurka (7)	4.68	4.69	5.14
Maris Mink (6)	4.59	4.63	4.97
Ark Royal (8)	4.58	4.71	5.09

[a]All mixtures comprised three cultivars; the range of sites is unique to each cultivar/mixture comparison.

with farming needs because it enables new, higher-yielding cultivars to be integrated into the system as soon as they become available.

Cultivar Mixtures and Yields

Convincing yield increases, particularly where mildew infection was reduced by host mixing, have been obtained for several mixtures over the past 3 yr. The most interesting and instructive data were obtained in 1978, when the system was examined on a larger scale than previously. Altogether there were 47 different comparisons in which the yield of a mixture, usually with three components, could be assessed against the yields of the components grown alone at the same site. The comparisons involved 37 mixtures of 25 cultivars from 12 resistance phenotype groupings.

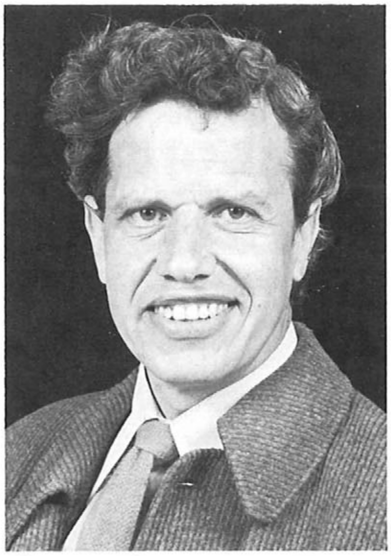
Overall, the average mixture yield was 106.5% of that of the means of the components grown alone; data from several sites where mildew was absent or unimportant were included. In two subsets of the data, the mean yield at seven sites with little or no mildew was 103% of the mean of the components, whereas the mixture yield over 10 sites with more mildew infection was 109% of the mean of the components. Because the data were obtained from a wide range of different types of trials, some with only single replicates of large (2-ha) plots, determining whether the yield increase was significant was not possible. However, the proportion of the comparisons in which the mixture yield was in excess was highly significant: 39 of the 47 comparisons equaled or exceeded the mean, and 26 of these exceeded the yield of the highest-yielding component of the appropriate mixture.

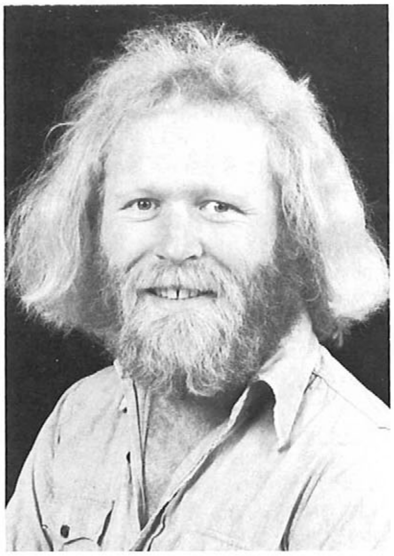
Comparisons of mixture yields with those of the highest-yielding individual components are not very illuminating, for two principal reasons. The first is a simple arithmetic problem: if the yields of individual cultivars are similar, a small proportional increase in the yields of mixtures may be sufficient to allow them to outyield the highest-yielding components. If there are large differences in individual yields, on the other hand, a large mixture benefit may still be insufficient to raise the mixture yield to that of the best component. Second, comparison of mixture yields with those of the highest-yielding components implies a retrospective judgment. If the use of mixtures can be justified only when they equal or exceed the highest-yielding components in monoculture, the highest-yielding cultivar for a particular field in a coming season must be predicted with accuracy. This may be realistic for some crops in some regions, but it is impossible for spring barley in the United Kingdom.

A subset of the 1978 data was analyzed in which each mixture contained at least one higher-yielding cultivar. The regression of the yields of 32 mixtures on the means of their components grown alone is shown in Figure 2; the mean yield increase of the mixtures over their components' mean was 6.5%, identical with that obtained from the whole data set.

The mean yields of the six cultivars over the sites at which each was grown is shown in Table 4, together with the yields of the mixtures at these sites and the expected yields of the mixtures, calculated as the mean of the components. Comparison among cultivars is not possible because each was grown at a different range of sites. The ranges and mean yields can be compared, however. Diversification, ie, growing three cultivars at each site rather than one, provides a mean yield similar to growing one cultivar at each site, but with less variability (0.15 t/ha and 0.41 t/ha, respectively). Thus, a farmer would be more likely to achieve a reasonable yield by growing three cultivars than by growing one. If he mixed the cultivars, however, the mean yield would be considerably increased and the probability of achieving that yield would be greater.

The reason why both diversification and mixing seem to be advantageous lies





Martin S. Wolfe

Dr. Wolfe is head of the Department of Pathology and Entomology at the Plant Breeding Institute, Cambridge, England. He joined the institute as a cereals pathologist in 1963 and was promoted to senior scientific officer in 1968, principal scientific officer in 1973, and senior principal scientific officer in 1978. He received his Ph.D. degree from Cambridge University in 1963.

John A. Barrett

Dr. Barrett is research associate in the Department of Genetics at the University of Cambridge, England. He received his Ph.D. degree from Liverpool University in 1975.

in the interaction between genotype and environment for each cultivar. Defining environments in which barley generally will yield poorly or well is possible. The precise way in which each cultivar responds to the range may be unique to that cultivar, however, and this appears to be true for our data. Small differences in cultivar response are difficult to measure, and variations in environment are difficult or impossible to predict. Therefore, a farmer cannot be sure which cultivar will be best suited to the local environment in a particular season. By mixing cultivars, he increases the probability of matching the conditions of the coming growing season and diminishes likely losses due to disease. With mixtures, yields will be more stable if disease does not occur and more stable and higher if disease does occur than with a single cultivar.

Apart from disease resistance characteristics, the main criterion for selecting mixture components is that they have the highest potential yield for the area. Pfahler and Linskens (11) came to a similar conclusion in studies of a range of oat multilines.

Several 1978 trials allowed comparison of the effectiveness of mixing cultivars with that of fungicide treatment in disease control. Six trials involving different mixtures and fungicide treatments all gave similar results. On the average, each method reduced disease by about the same amount and increased yield by 7%. Fungicide treatment of the mixtures reduced disease still further, but an additional yield increase of 4% was not significant.

Neither mixing cultivars nor applying foliar fungicide sprays reduces disease at the beginning of the season. We are currently studying, therefore, application of fungicides to the seed of mixed cultivars in the hope of combining early disease control from seed-applied fungicide with late control due to crop heterogeneity. Another advantage of seed-applied fungicides is that they can be directed at specific components of the mixture, eg, one component can be treated one year and different components in subsequent years. Because this effectively changes the resistance characteristics of different cultivars in different years, the obstacles facing the pathogen are multiplied and the emergence of complex races may be further delayed.

Advantages of Cultivar Mixture System

On a small scale, heterogeneous host crops provide disease control and a more stable yield production. For large-scale effectiveness, the direction of selection must be altered continually and the spread of complex pathogen races must be delayed. Mixing appropriate cultivars provides a simple means of achieving these ends. It is a flexible approach allowing inclusion and exploitation of new cultivars as they appear, without creating difficulties in the legislation of cultivar registration. The system does not limit the plant breeder as more complex systems do, such as selecting for polygenic resistance or developing near-isogenic lines for multiline production. In addition, cultivar mixtures carry, or can be arranged to carry, resistance to diseases other than the major target disease.

Large-scale screening of mixtures for relative performance seems unnecessary and would be impractical, since the total number of potential cultivar combinations may be large. The number of combinations of resistance groups is given by

$$ {}^{n}C_{r} = \frac{n!}{r!(n-r)!} $$

where n is the number of resistance groups available and r is the number of groups required in each mixture. In addition, if each resistance group has several different cultivars, the number of cultivar combinations will be the product of the total number of cultivars in each of the relevant groups. For example, if six resistance groups are available but only three are required for a mixture, the total number of resistance group combinations will be 20. However, if four cultivars are available within each group, the possible total number of cultivar combinations will be 1,280. Such a number would be impossible to test but would allow considerable scope for selecting cultivars to control secondary diseases, to meet regional environmental requirements, and so on.

Such flexibility is beyond the scope of the classic multiline approach. The current trend in multiline development, following the pioneering work of breeders and pathologists such as Norman Borlaug and Artie Browning, is more practical in that less attention is being paid to uniformity of component lines. No more than two or three backcrosses to a recurrent parent are made, and selection in the resulting progeny is toward a general plant type with, of course, heterogeneous disease resistance. At the Plant Breeding Institute, we are developing material in a similar way to produce more closely matched mixture components; our aim is separate registration of the lines so that the final composition of the mixtures can be easily changed.

The great advantage of the classic multiline is that it yields a uniform product, which is desirable for industrial processing. A cultivar mixture will, of course, be more heterogeneous in this respect, but considering the large number of characteristics that make up quality, mixed crops may prove to be more uniform than pure cultivars when taken from a range of environments.

Our experience has been limited mainly to control of a major disease of a single crop in the United Kingdom. The model can be generalized, but the system is not necessarily applicable to all problems of disease control and in some cases may not be the most practical solution. A major development of potential importance is the use of heterogeneous wheat crops to control the three major rust diseases in India, where stability of production is vital (M. V. Rao, *personal communication*). A similar system could be of value in controlling blast, a principal limitation to increased rice production in Asia.

So far we have considered only the possibilities for within-crop diversity. Where uniformity of the end product is less of a problem, as in the feed market for cereals, might we not consider mixing different cereal species in the field? For example, barley/oat mixtures consistently outyield the mean of the components. Considerably more diverse mixtures have been widely used in subsistence agriculture for thousands of years. Many are not suitable for high-yield production because of the widely different needs of the components in terms of fertilizer, machinery, and other inputs. Nevertheless, some intercrop mixtures merit consideration, and this approach is being investigated in India, Africa, and elsewhere.

Perhaps we have gone too far along the road to crop uniformity in the interests of industrial processing. To support and exploit this uniformity, the system of agricultural production has had to attempt to control various features of the environment. A more biologically sound approach may be to produce heterogeneous crops that are better able to exploit the range and counter the limitations of the environment, at lower cost.

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