TOPIC 5B

PLANT BREEDING STRATEGIES FOR CROP PROTECTION

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PLANT BREEDING OR PESTICIDES TO NARROW THE YIELD GAP ?

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ABSTRACT

The process of weighing resistance among all other yield factors by commercial, public and so-called international breeders is discussed. Special attention is paid to the feasibility and willingness to breed for durable resistance, which over all appears to be neglected by all three categories of breeders. Insufficient and instable resistances linked with changes in the agricultural technology have caused an enormous and continuing increase of world-wide pesticide usage.
Alternatives and their consequences for breeders, farmers, pesticide producers, consumers and the environment are indicated but it is concluded that at least on the short-term, pesticide sales are not threatened by resistance breeding.

Plant breeding is an activity intended to produce new superior varieties that will generate greater farm (or forestry) income and thus greater returns also to those developing the new varieties. Pesticides and their development and use have exactly the same ultimate intent. The two areas coincide for control of diseases and pests but not (yet) for weeds.

When the positions of disease and insect resistance in breeding programs are examined, it becomes clear that there are many conflicting forces influencing their importance.

On a world-wide basis there are three types of breeding programs. 1) Commercial, 2) Public, and 3) International.

COMMERCIAL BREEDING

The commercial breeder must invest limited resources for the immediate target of a new variety with better yield or quality potential than some already existing variety. Any deviation from superior yield/quality will result in being overtaken by someone else's new variety and the loss of support for continuation.

Since there are many factors influencing yield, how does one balance the relative effort on disease and insect resistance among all the other components influencing yield ? This is a very complex issue requiring many guesses and much judgment of interactions. The points which the commercial breeder and his company consider include:

1. Importance of diseases and insect pests on existing varieties in the target seed-sale area.
2. Cost, effort, and effectiveness of existing disease and insect control measures.
3. The acceptability of these control measures for producers and consumers, for the public, in terms of potential health and environmental hazards.
4. Probability that advertisement of a "resistance" for a new variety will generate seed sales additional to the yield/quality attributes of the new variety.
5. Degree of "durability" of resistance desired in terms of future seed sales of newer varieties.

6. When a seed company is owned by a chemical company, a consideration of sales of protectants vs. sales of "resistant" varieties is obviously not an unconfounded consideration in planning the breeding strategy.
7. Probability of obtaining higher levels of disease or insect resistance in a "better" variety with the resources and time available.
8. Additional cost in time and money of developing the desired level of resistance.

Obviously, to judge each of these points and to come up with an integration that will result in the most optimum strategy is a subjective activity and different people and organizations will follow different paths. The points raised also require a lot of background knowledge for intelligent analysis. The complexity and probability for error speak to the need for different people and different companies, so that different strategies will be followed and competition will sift the winners. The problem will be exacerbated, however, if too little diversity of thinking exists and this is combined with too much competition, channelling everyone into the same, and a losing strategy. Farm production may suffer and great losses may occur. This is what happened in the great 1970 maize blight epidemic in the USA, with some 6 billion dollars' loss to normal production. Someone made a wrong turn and everyone else in the commercial seed industry copied him.

This example says that commerical breeders can be too short-sighted and too channelized in terms of how they and their bosses weigh the future. Societies' needs may be short-changed. With plant breeders'rights legislation in Europe, and now in the USA, this thinking is being extended to crops formerly bred mainly by public breeders. Thus, there becomes less support for public breeders, and lobbying exists to trim the role of public breeders in breeding crops. They are now supposed to do "background" research and breeding and "enhance the germplasm base". These changes are very actual in the UK now, with a structural reorganisation of the plant breeding institutes and their relation to the breeding industry.

PUBLIC BREEDING

An examination of the 8 points listed above that dominate decision-making of commercial breeders reveals that only some need apply to public breeders. Public breeders are those supported by public funds or by altruistic foundations. Moreover, public breeders can operate with a more protracted time frame, altering how one weighs the points. This is because rewards may accrue in areas different from or additional to seed sales and acreage covered. Students may be trained; scientific papers written; interesting hypotheses can be explored; greater risks can be taken (in terms of research approaches); and longer-term goals can be supported; benefits to society can be weighed more heavily; greater diversity of scientific enquiry can be followed. However, by retaining as one reward, a potential new variety, the research can be influenced in some individuals to be not only esoteric.

Where public breeders are at Universities and governmental institutes the possibility of linking with pathologists, entomologists, and crop physiologists exists, especially through graduate student or postdoctoral projects. Such linkage should enable exploration of methods and strategies on disease, insect and stress resistance in relation to breeding and genetics. New hypotheses that relate epidemiology of disease and insect pests to genetics of resistance and susceptibility can be explored. Efforts to develop resistance that remains durable and, thus, does not breakdown

can be explored. Efforts to understand pathogen population genetics and dynamics in relation to introduction of new varieties can be made.

All these things can be done, but are they? In general, hardly. Unfortunately, too little active research is underway to try to understand pathogen and pest dynamics in relation to selection of new crop varieties. Why does one develop and promote plant A as a new variety instead of plant B? How much concern or information is there in making such a decision that there will be less disease or less pest attack with the one rather than the other ? Often very little. If there is concern there is often little information applicable to the basic question of how the single plant performance will compare disease-wise with a whole field of the same genotype. There is even less information on how to tell whether the level of resistance seen will remain that level in the future farmer fields. In general, the breeder and the pathologist / entomologist are both in a quandary as to how to obtain "durable" disease or insect resistance. Controversy over horizontal and vertical resistance -- ideas now over 20 years old -- still rage. (Horizontal resistance is supposed to be non-race specific and thus is not likely to change markedly with exposure to other races or biotypes). But how does one breed for it and who is doing research on this question ? Anyone ? Hardly.

So it appears that public breeders, although having the potential of exploring new avenues of research to improve the methods and strategies that would increase disease and insect resistance, and the durability of this resistance, are still doing little in this area.

Part of the problem is due to the close relationship between private and public breeders and the nature of crop improvement itself. The highly-bred varieties have unique arrangements of genes honed to a fine adjustment to an overall environment. If disease or insect resistance is insufficient or if it "breaks down", the breeder will still wish to stay with the highly-bred varieties, adding only a major gene for resistance. This means "inserting" a new gene, usually from a poor agronomic type, and backcrossing to the good type several times. This takes time, but generally less time than attempting to breed for horizontal resistance by opening up the germplasm base and intercrossing many parents and recrossing selections in a recurrent selection program. Thus, a public breeder who develops horizontal or durable resistance in a variety that is perhaps also less-than-the best in yield finds that the commercial breeder will not use it since this resistance is often due to several or many genes with small effects that cannot be manipulated easily in a crossing and selection program. The present direction of some public breeders -- towards "germplasm enhancement" by broadly based resistance -- may therefore be a dead end if this depends on many minor genes.

INTERNATIONAL BREEDING

The "international breeder" is a breeder at an international institute such as at IRRI, the rice institute in the Philippines, CIMMYT in Mexico, IITA in Nigeria, etc. Such breeders and the international institute in general wield an enormous influence on that happens in third world agriculture. They are responsible for the "green revolution" and for a great deal of training of third-world agricultural scientists. Their perceived purpose is tho shift low-productivity peasant agriculture into greater

productivity by developing varieties with greater yield potential expressible with higher inputs and higher technology in general. Thus there is a union of varietal improvement with a sharp increase in need for technological improvements. Great yield increases have occurred in rice and wheat, followed by periodic varietal collapses due to the rise of one or another insect pest or disease. An increase in pesticide usage has resulted, with cyclic introduction also of "new" resistance genes.

Because the international breeders rewards are largely related to the area planted to his new varieties, forces are set in motion for genetic uniformity on a grand scale, thus creating gigantic monocultures with all their attendant hazards. Besides varieties are moved into new pathosystems, increasing the chances for new problems, not perceived in the original development of the varieties. Continued success is perceived as solving the new problems, which were actually created by a previous strategy and accomplishment.

Thus, the international breeder, and his protégés in Third World national programs, comes to resemble the commercial breeder, whose rewards are similarly based on area planted (but in this case measured by seed sales). The cycle will be first to improve yield potential with plant type changes; second to protect this yield potential with pesticides; third, (as new problems arise) to introduce single major resistance genes to provide cheaper protection; and fourth, as these "break down", introduce new ones, protecting the crop meanwhile with pesticides. The cycle is set for the treadmill linking technology and breeding that characterizes European or American agriculture.

This pattern has resulted in increases in worldwide pesticide usage (including herbicides) from $0.85 billion in 1960 to $2.7 billion in 1970 to $13.3 billion in 1982. Pesticides for disease and insect control are now about $7.3 billion in worldwide sales. Discounted for inflation by one formula these changes represent a doubling every 10 years. An annual growth rate of these is projected at 4-5%. It should be obvious that, as measured by these figures, plant breeders overall are not solving disease and insect problems with resistance. Moreover, strategies and large investments by chemical companies are predicated on the assumption that the future will reflect the past in this regard.

ALTERNATIVES

Are there any alternatives? Some of us think there are, but to implement them is beset with many difficulties -- technical and political.

But what are these alternatives ? Details would take a book but in brief:

1. Avoid deploying varieties into new environments differing from the area where they were bred and selected in having different pathogens and pests or environmental influences affecting their buildup.
2. Point one means having more localized breeding programs for local problems and environments.
3. Point two payoff requires developing an increased understanding of the local pathosystem, enabling the development of resistance sufficient in that system to preclude economic damage.
4. Point three will require improvement in methods to detect and select for the level of resistance required, in segregating populations of diverse breeding material.

Figure 1

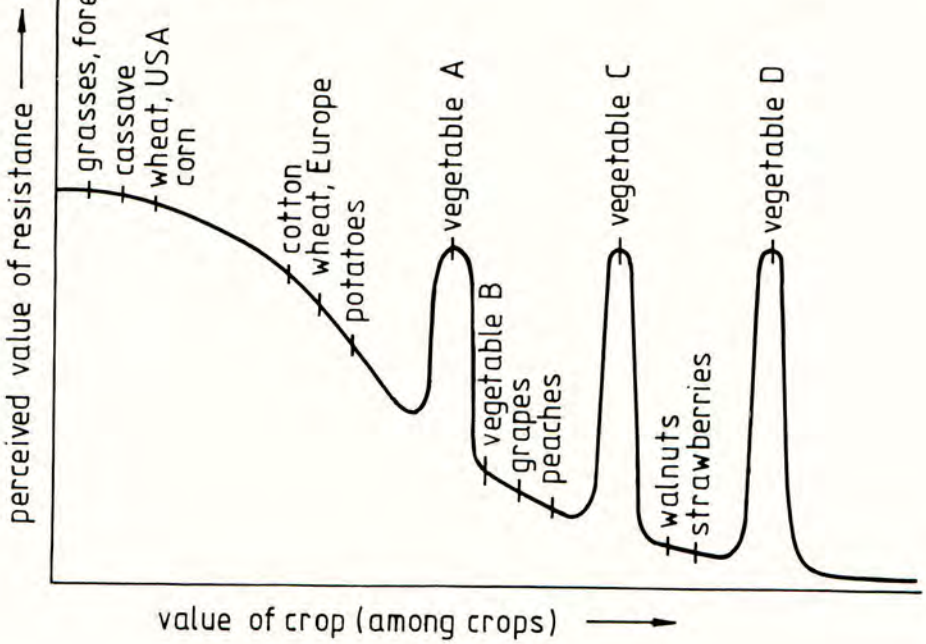
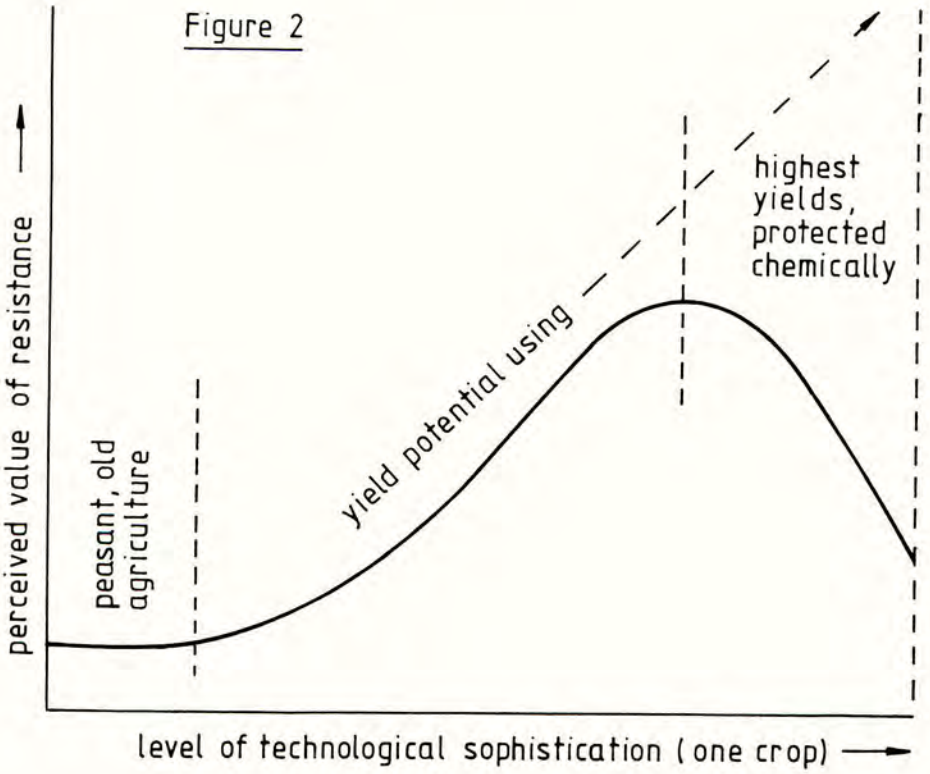


Figure 2



5. Fundamental to overcoming the breakdown of resistance, (the phenomenon underlying the pessimism that resistance is never really going to be successful), is the need to change breeding strategy toward breeding for horizontal resistance. This means developing new strategies and methods that enable selection of small increments in resistance, collectively due to polygenes, just as is yield. A fundamental controversy exists today on whether this can be done.
6. Breeders, pathologists and entomologists should finally appreciate the value of partial resistance as a major attribute to suppress the population development of disease and insect pests. Partially resistant varieties fit, therefore, perfectly in integrated control or integrated production systems aiming at a fine tuning of all production and protection factors. Thus fertilizer and pesticide usages are minimized, natural resources not exhausted and agro-ecosystem balances safeguarded. Such integrated agriculture is focussed on yield optimization instead of yield maximization.

Successful pursuit of each and all of the points above will require increased investment in research and development in specific ways. Changes in thinking and in education will be required. Such changes can occur only if some of those in a position to control funds and policy believe that changes really will make a difference.

What are the forces involved? Success would probably lead to the following results:
1. Less effort and cost to the farmer.
2. Greater and more stable productivity and thus potentially lower prices for product or food.
3. Increased cost of plant breeding.
4. Decreased seed sales for a specific variety.
5. Decreased breeder rewards as measured by varietal use.
6. Decreased sales and use of pesticides.
7. Decreased environmental pollution.
8. Increased cost in basic research.
9. Increased employment for and deployment of agricultural researchers.
10. Increased effort to deviate from existing paths.

We leave it to you to judge how these forces interrelate and which of them are the more powerful. Although we believe greater resistance that is more durable could be developed, and that this would be a desirable overall objective in the public interest, we recognize the many conflicting forces that inhibit this goal.

Some of those that affect perception of the value of resistance are illustrated in figures 1 and 2. Above and beyond perception problems and the conflicting forces inhibiting change are the technical difficulties.

Some crops are perennials and difficult te breed; breeding cycles are long, even 2 to 6 per professional career, especially for forest or some orchard trees. Quality considerations inhibit resistance breeding. Many high quality perennials are still ancient varieties selected in pre-science: grapes, peaches, apples, pears, bananas, pineapple. Sometimes quality standards have reached so-called cosmetic levels, which have no relation with the real intrinsic quality of the product. This is especially true for fruits and ornamentals and inhibits the development and use of alternative strategies of control, including horizontal and partial resistance.

Some pathogen and pest systems are highly unstable and their shifting vertical pathogenicity presents great difficulties in breeding for durable resistance.

Will genetic engineering make a difference ? Plant breeding of crops is already highly refined. Genetic engineering deals with specific individual genes. It is such individual genes which are already suspect as the cause of resistance breakdowns. Manipulating them through genetic engineering is hardly a solution to durability of resistance. However, for crops difficult to breed, or with long breeding cycles, genetic engineering has more promise. Also, for crops with a history of difficulty of combining high quality and resistance, genetic engineering offers promise. Since most plants are already resistant to most potential pathogens, who can say whether molecular biology may not show us how to find and insert that type of non-specific resistance into our susceptible crop varieties ?

In summary, it appears that pesticide sales are not threatened by crop resistance breeding at present. It also appears that greater efforts to develop more resistance that is more durable would be in the public interest. Long range productivity of our forests and crops should rely more heavily on increased knowledge of pathosystems and the means to tip the balance in favor of plant health. Man, as a species, would benefit. How to bring this about, in terms of changes in policy and research support and in education and attitudes is a major challenge.

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GENETIC ENGINEERING AND TISSUE CULTURE IN CROP PROTECTION

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ABSTRACT

Many different *in vitro* procedures of plant tissue culture and molecular biology are offering new approaches to plant breeding. *In vitro* selection, somatic hybridization and genetic transformation are useful to genetically manipulate plant cells for improved disease resistance. Experimental constraints such as the lack of plant regeneration from protoplasts of important crop species and our poor knowledge of the molecular and genetic basis of polygenic agronomic traits still pose limits to applied uses of these techniques at present. Major advances in these areas are needed before tissue culture and genetic engineering technologies will significantly contribute to agricultural improvement.

INTRODUCTION

In the past decades plant tissue culture techniques have undergone an exciting development and their potentialities for studies in plant pathology and, ultimately, crop improvement have soon been recognized (Thomas *et al.* 1979). Over the years, an ever increasing collection of techniques has accumulated (Reinert & Bajaj 1977; Ingram & Helgeson 1980; Thorpe 1981) some of which already constitute integral steps in plant breeding programs. Other techniques, such as the more sophisticated approaches of *in vitro* selection, somatic hybridization and genetic engineering, which appear as pure rather than applied sciences today, show a tremendous potential for future application in crop improvement.

Progress has been extremely fast in the area of recombinant DNA research during the past decade. Great efforts have also been made in the molecular analysis of higher plants. These studies have opened exciting new perspectives for the directed genetic manipulation of crop plants through *in vitro* techniques (Cocking *et al.* 1981). Aspects of crop protection rank within the highest priority of genetic engineering projects.

In this article I will review tissue and cell culture approaches used to effect genetic changes that could be advantageous in the breeding of plants exhibiting improved resistance to herbicides, diseases and pests.

PLANT TISSUE CULTURES IN PLANT BREEDING

The following paragraphs will briefly summarize some established tissue culture techniques that assist plant breeders in their breeding programs, particularly those concerned with the improvement of resistance.

Plant tissue cultures referred to in this article constitute a wide range of different materials, materials differing in their genotype, their structural complexity, state of differentiation, texture, homogeneity, cellular or tissue organisation and many other characteristics. Culture techniques available for a given plant species determine the approaches that are fea-

sible at a given time. In assessing what has been achieved it is important to consider the nature of the cultures used.

Ovule and embryo culture

Ovule and embryo culture (Raghavan 1977) in many cases allow to rescue hybrid embryos from wide crosses which would otherwise fail to complete their development due to a post-zygotic incompatibility reaction. The culture medium replaces the malfunctional endosperm in supplying the necessary nutrients and growth factors. This technique can be particularly useful to achieve introgression of resistance genes from more distantly related species, for instance weeds, into crop plants.

Shoot tip culture

Excised shoot tips or buds regenerate into complete shoots and plants *in vitro* (Conger 1981) thus allowing the rapid clonal propagation of useful plant genotypes. Since it has been observed that meristems usually do not contain pathogenic microorganisms, viruses in particular, the culture of excised meristems has found widest application, for instance with berry crops, for the eradication of virus diseases (Wang & Hu 1980) and for the rapid propagation of virus-free mother plants.

Haploid production by anther and isolated microspore culture

Haploid individuals are found rarely among diploid populations. Haploids can also be generated, sometimes at high frequency, by means of specific crosses (Kasha 1974). The culture of anthers or even isolated microspores provides another method now available in many crop plants including the cereal species (Nitzsche & Wenzel 1977). Although the efficiency of these latter techniques will have to be improved for commercial application, haploids provide a number of distinctive advantages to the breeder. These include simpler segregation patterns and thus significantly lower numbers of individuals among which a desired genotype can be found; immediate expression of recessive traits; easy and fast attainment of homozygosity. The more gene differences are involved, the more attractive are haploids in the breeding process.

Androgenetic haploids are progressing into various breeding programs (Wenzel *et al.* 1979; Wenzel & Uhrig 1981). Several new cultivars of tobacco, rice and wheat, now appearing on the market, have been generated through anther culture, indicating the feasibility and utility of this technique. Besides accelerating breeding programs, haploid plants can be useful as a source of haploid cell cultures for *in vitro* selection.

SOMACLONAL VARIATION OF TISSUE CULTURE-DERIVED PLANTS

The relative ease at which numerous plants can be regenerated from cultured explants, tissues, even isolated cells and protoplasts represents a major significant contribution of *in vitro* techniques to horticulture and plant breeding (Conger 1981). The clonal vegetative progenies thus obtained were assumed to be genetically homogenous and uniform. Aberrant phenotypes have often been regarded as curiosities rather than interesting novel materials.

The potential usefulness of somaclonal variation for crop improvement has first become apparent in sugarcane. Resistance to the Fiji virus disease,

downey mildew *(Sclerospora sacchari)* and eyespot disease *(Helminthosporium sacchari = Drechslera sacchari)* was found among plants regenerated from tissue cultures derived from susceptible sugarcane cultivars (Heinz *et al.* 1977; Larkin & Scowcroft 1983). The incidence of resistant lines was not dependent upon a specific selection during the tissue culture and plant regeneration phases. About 70% of the somaclones vegetatively propagated from resistant plants derived from tissue culture were again resistant to *D.sacchari* (Larkin & Scowcroft 1983). A second tissue culture cycle yielded regenerants exhibiting different degrees of resistance, 20% being more resistant and 40% being less resistant than plants from the primary culture. Resistant somaclonal lines generated by *in vitro* methods are now being introduced into programs for sugarcane improvement (Heinz *et al.* 1977; Liu 1981).

Shepard *et al.* (1980) have proposed a protoplast and tissue culture based scheme for the 'intracultivar improvement' of potato by use of somaclonal variation. Great variability was found for various traits in over 10'000 somaclonal plants ('protoclones') derived from cultured mesophyll protoplasts of the cultivar 'Russet Burbank'. About 1% of the somaclones were more resistant to *Alternaria solani* toxin and also showed field resistance to early blight. Somaclones resistant to late blight *(Phytophthora infestans)* were found at a frequency of 2.5% and some of the clones were resistant to multiple races of this pathogen (Matern *et al.* 1978; Shepard 1981).

A wealth of possible causes have been discussed of somaclonal variation (Chaleff 1981; Larkin & Scowcroft 1981) including aneuploidy, chromosomal rearrangements, gene amplification, somatic crossing over. It appears that the situation may differ, and must be clarified anew, in each individual case. The data available at present do, however, suggest a great inherent potential of somaclonal variation at least in vegetatively propagated species, and this is currently being exploited in many institutes around the world.

IN VITRO SELECTION FOR RESISTANCE

The addition of lethal concentrations of a phytotoxic compound to the culture medium has been proposed as a straightforward tissue culture approach in order to select for cells exhibiting toxin, antibiotic or herbicide resistance. Cells surviving the selective conditions should then be induced to regenerate into resistant plants. In view of improving disease resistance, host-specific toxins produced by fungal pathogens appear to be good candidates as selective agents (Brettell & Ingram 1979). If a toxin plays a role in pathogenicity, resistance to the toxin should result in some resistance to the pathogen itself. Such toxins (Yoder 1980) are known of *Drechslera maydis* Race T (southern corn leaf blight), *D.victoriae* (victoria blight of oats), *D.sacchari* (eyespot disease of sugarcane), *Periconia circinata* (Milo disease of sorghum), *Alternaria alternata* (stem cancer of tomato), *A.solani* (potato early blight). Other pathogens, like *Pseudomonas tabaci* (tobacco wildfire disease), *P.phaseolicola* (halo blight of bean) and *Phytophthora infestans* (potato late blight) produce unspecific toxins. In many diseases there seem to be no toxins involved, in other cases the nature of the toxin has not yet been elucidated and culture filtrates of the pathogen have then been employed for selection instead of purified toxins.

Maize plants resistant to southern corn leaf blight caused by the fungus *D.maydis* Race T were recovered from tissue cultures of susceptible T cyto-

plasm maize following recurrent sublethal exposure to T toxin (Gengenbach *et al.* 1977). Brettell *et al.* (1980) obtained resistant plants even in the absence of selection. The resistant plants also exhibited restored male fertility, and both characters were shown to be maternally inherited and coded for by mitochondrial genes (Gengenbach *et al.* 1981; Kemble *et al.* 1982). The inability, in these experiments, to separate the T type male-sterility from disease susceptibility limits their agricultural value but does not render the underlying *in vitro* approach unvalid. Selection of desirable traits represents a first step towards crop improvement and must be followed by careful analysis of the other agronomic features of the selected material.

Sacristan (1982) exposed callus and embryogenic cultures of rape *(Brassica napus)* to culture filtrate from the fungus, *Phoma lingam*, causative pathogen of the black leg disease of *Cruciferae*. Out of a total of 63 plants regenerated from selected embryogenic cultures treated with culture filtrate, two were graded as resistant and 12 as tolerant upon infection with *Ph. lingam*, whereas 49 were susceptible. Three tolerant plants were also obtained among 64 control plants from untreated cultures. A preliminary analysis of limited progenies suggested a genetic nature of the selected resistance.

Culture filtrate from *P.infestans* was used by Behnke (1979) to select resistant potato callus. Regenerated plants, as well as secondary callus cultures derived from them, were found resistant to culture filtrate whereas plants from unselected callus were susceptible. These results indicate stable vegetative transmission and the expression of resistance both *in vitro* and *in vivo*. When leaves of plants from selected callus were inoculated with the fungus, they showed somewhat smaller lesions than unselected plants but sporulation of the fungus was not inhibited (Behnke 1980a).

The same approach was employed in attempts to select potato callus resistant to culture filtrates from *Fusarium oxysporum* (Behnke 1980b). 35 calluses were selected, and leaves from some of the regenerated plantlets were less sensitive to culture filtrate than were control leaves. The results remain inconclusive in that fully sensitive as well as slightly more tolerant shoots were produced from the same selected callus, indicating either instability of resistance or chimericity of the callus material. A genetic analysis of resistant plants was not undertaken, perhaps because sexual crosses in potato are difficult.

Complex cultures such as the materials used in the above studies suffer from a number of serious disadvantages when used for *in vitro* selection purposes. In complex cultures, not all cells are equally exposed to a mutagenizing or a selective agent. Sensitive cells within larger aggregates may survive and eventually give rise to chimeric tissues from which only susceptible or a mixture of resistant and sensitive plants may be regenerated. The need to transfer individual pieces of tissue or callus by hand greatly limits the number of experimental units from among which selection is made.

These disadvantages can be circumvented when using cultures of protoplasts or single cells (Thomas *et al.* 1979). Very high numbers in excess of 10^7 cells can thus be handled and screened for useful mutants, each of them potentially capable of forming a whole plant. This "microbial" approach, selection *in vitro* from among millions of cells, suggests a potential practical alternative to the screening of hectares of plants in the field. It is clear,

though, that selection can only be operative on traits that are expressed in cultured cells.

Using this approach, many cell lines have been selected which exhibit resistance or increased tolerance to various antibiotics, antimetabolites, herbicides, toxins, stressful concentrations of sodium chloride and other agents (Chaleff 1981). Due to a loss of their regenerative capacities the majority of these variants exist as cell lines but not as regenerated plants. Sexual cross analysis to elucidate the genetic nature of a variant has therefore been limited to but a few of these lines. Complementation analysis following protoplast fusion (Harms *et al.* 1981) may provide a somatic cell genetic approach to this problem.

Resistant variant cell lines have contributed to our understanding of the biochemical and physiological alterations involved, but complete resistant plants will be required if *in vitro* selection techniques are to effect a significant contribution to agriculture and crop improvement. Examples are still scarce of resistant plants that were regenerated from selected cell cultures. Carlson (1973) subjected mutagenized 'haploid' tobacco cells to methionine sulfoximine, then thought to be a structural analog of the tabtoxin produced by *Pseudomonas tabaci*, the causative pathogen of tobacco wildfire disease. Plants were regenerated which exhibited enhanced resistance to the disease. Preliminary studies on F_2 progeny showed a complex inheritance of the trait.

Plating cells of tobacco on a medium containing 500 μM of the herbicide picloram yielded resistant colonies and finally plants that transmitted picloram resistance either dominantly or semidominantly to their sexual progeny (Chaleff & Parsons 1978; Chaleff 1981). Tobacco cell cultures tolerant to paraquat were selected and subsequently regenerated into plants, one third of which were found tolerant in a leaf disc assay (Miller & Hughes 1980). Recent crosses suggested the sexual transmission of paraquat tolerance in these lines. In similar experiments, Thomas and Prat (1982) isolated tolerant lines from tomato cells exposed to toxic levels of paraquat. Secondary callus cultures, derived from plants that were regenerated from tolerant lines, showed growth at concentrations non-permissive to wild type cultures. Callus derived from the sexual progeny of tolerant lines varied in growth response but at least a proportion of them had retained a tolerant phenotype, indicating sexual transmission of paraquat tolerance.

Exposure of tobacco cells to toxic levels of chlorate yielded resistant lines lacking nitrate reductase and auxotrophic for reduced nitrogen (Müller & Grafe 1978). Genetic studies on plants regenerated from chlorate resistant cells have shown chlorate resistance to result from a double, recessive mendelian mutation. Although there is no agricultural value of plants being resistant to a herbicide and lacking a key enzyme of nitrogen assimilation, these studies nicely illustrate the kind of *in vitro* approaches used to select for resistance in plant cell cultures. Ever increasing costs for the development of new herbicides and breeding for disease resistant cultivars are demanding unconventional alternatives. Introducing resistance into a crop species by *in vitro* genetic manipulation and selection is an appealing strategy.

SOMATIC HYBRIDIZATION BY PROTOPLAST FUSION

The past decade has seen the emergence and the rapid development of protoplast technology and somatic cell fusion in higher plants. Protoplasts of many species can now be cultured to give whole plants. Fusion of protoplasts even from diverse species can be induced by polyethylene glycol. Such promiscuity has led to the presumption that protoplast fusion would allow novel plants to be created beyond the limits of sexual cross incompatibility. In fact, an increasing number of interspecific and intergeneric somatic hybrid plants have been produced which do not normally arise from sexual crosses. There is evidence, however, that various forms of somatic incompatibility may interfere with the development of functional and competitive somatic hybrids between remote species (Harms 1983). For agricultural purposes, somatic hybrids of not too distantly related species will probably be more important in the short run than remote hybrids.

First attempts to use protoplast fusion as a method to introduce disease resistance from a resistant species into a susceptible cultivar have been reported by Evans *et al.* (1981). They fused protoplasts from *Nicotiana repanda*, *N.stocktonii* and *N.nesophila* with protoplasts of the cultivated tobacco, and somatic hybrid plants were selected of the latter two combinations. Hybrids *N.tabacum + N.nesophila*, when inoculated with tobacco mosaic virus, showed local lesions which are typical for the hypersensitive response of *N.nesophila*, and there was no systemic infection as in tobacco. The reaction of these hybrids to race 1 of *Phytophthora parasitica* var. *nicotianae*, to which tobacco is susceptible whereas *N.nesophila* is resistant, remains to be examined. The results of Evans *et al.* (1981) indicate that disease resistance can be transferred by protoplast fusion and become expressed in somatic hybrid plants. Sexual hybrids between these species have been produced by Reed and Collins (1978) using ovule culture but protoplast fusion probably was the more efficient method.

Schenck and Röbbelen (1982) succeeded in resynthesizing amphidiploid *Brassica napus* from its ancestors, *Brassica oleracea* and *B.campestris*, by protoplast fusion. Using different *Brassica* varieties as parents, it now seems possible to extend the presently limited genetic germplasm resource of rape, and to introduce new disease resistance traits by means of somatic hybridization.

There are numerous examples where protoplast fusion should provide unique prospects for the creation of hybrids between a crop cultivar and a wild relative which possesses desirable resistance genes. Such somatic hybridization is confounded, at present, by the still low number of plants whose protoplasts can be cultured and regenerated. Plant regeneration from protoplasts is possible (in addition to the model plants tobacco, *Petunia* and *Datura*) in potato, carrot, eggplant, rape, asparagus, citrus and *Cichorium*, but it is lacking in sugarcane, beets, soyabeans and, most important, all major cereals (Harms 1982). Great efforts must be concentrated on developing the necessary protoplast and single cell culture technologies of important crop plants before they can efficiently be used in crop improvement.

PLANT CELL TRANSFORMATION

Most often plant breeders aim to transfer but a few interesting genes in their crosses, and repeated cycles of back crossing and selection serve to achieve this goal. Although protoplast fusion combines whole genomes at first, the resulting somatic hybrids can segregate, via recombination, somatic crossing over and chromosome elimination (Harms 1983), to ultimately give addition or substitution lines similar to those known from classical plant breeding. The most direct approach would seem plant cell transformation, a technique that attempts to transfer isolated genes directly into a recipient cell (Cocking *et al.* 1981).

A generalized strategy for plant cell transformation requires the construction of a genetic vehicle composed of the desired genes flanked with the necessary regulatory regions, promotor and replication sites, and selectable markers which allow for its cloning in a bacterial system and secure stable incorporation, multiplication and expression in the host cell. Among the potential genetic vectors under construction (Howell 1982), the Ti-plasmid of *Agrobacterium tumefaciens* has been studied in most detail (Schell *et al.* 1982). Both *in vivo* and *in vitro*, *A.tumefaciens* transforms dicot plant cells into crown gall tumor cells. This is accomplished by the transfer, incorporation into the host genome, and finally expression of T-DNA, a specific fragment of the Ti-plasmid. For its use as a DNA vector, the Ti-plasmid is being genetically engineered to eliminate its oncogenic properties while maintaining its infectivity. Ti-plasmid has been delivered to plant protoplasts in various ways and it can eventually become expressed as is evidenced by the hormone independent growth and opine synthesis of transformed cells. Coding sequences inserted to the T-DNA, such as the bacterial transposon Tn7 which encodes streptomycin, spectinomycin and trimethoprim resistance, were shown to be transferred unchanged and physically incorporated into the plant DNA (Hernalsteens *et al.* 1980). Most recently, transcription and functional expression of antibiotic genes inserted into modified T-DNA has been demonstrated (Herrera-Estrella *et al.* 1983a, 1983b).

The way now seems open to genetically manipulate plant cells via Ti-plasmid-mediated transformation. There are several major hurdles, however, that must be overcome before plant cell transformation will be able to contribute to the solution of practical problems in plant breeding. Stable incorporation, mitotic and meiotic transmission and phenotypic expression of transferred foreign DNA are required. Selective systems must be available to allow specific recovery of the rare transformant cells from among the many that are unchanged. When transferring genes for disease or toxin resistance, these themselves may provide useful selective markers if expressed at the cell level. In other cases it will be necessary to cotransfer suitable markers, and bacterial genes coding for antibiotic resistance are the major candidates for this.

The most serious obstacle to the use of directed gene transfer is our poor knowledge of the molecular biology and the complex genetics of genes that encode disease resistance. Since resistance to so many diseases is under polygenic control, and since these genes are often scattered over several chromosomes, transfer of a single DNA sequence may not be very helpful. At present, none of the known plant genes encoding disease resistance has as yet been isolated and characterized by biochemical and molecular biological tech-

niques. Most plant characters of agronomic interest, however, are quantitative in nature and are controlled by polygenic complexes which are likely to be much more difficult to transfer.

CONCLUSIONS

This review has aimed at assessing the novel strategies of plant tissue culture and genetic engineering that are now becoming available for the improvement of crop plants. Besides the unique prospects that these *in vitro* approaches are offering, this discussion has also elucidated the gaps in our present knowledge that will have to be filled by forthcoming studies. In view of the great inherent potentialities of these techniques for crop improvement and also the urgent necessity to exploit all promising approaches, these efforts seem justified and well worthwhile.

Conventional plant breeding thus far has been tremendously successful in providing us with improved cultivars of crop plants. Patience, endurance, obstinacy, and a long breath have been characteristic qualities of plant breeders in pursuing their aims. These qualities are also needed now that plant breeders, plant tissue culturists and molecular biologists are combining their efforts to bring into reality the promises that plant tissue culture and genetic engineering technologies hold for us.

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5B—S3

EXPLOITATION AND CONSERVATION OF GERM PLASM FOR CROP PROTECTION

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ABSTRACT

All cultivated crops display disease resistance. Chemical control can only add to this resistance, not replace it. Rapid increases in the efficiency of crop production have led to an erosion of genetic resources and a narrowing of the genetic base of crops, not least for disease resistance. To ensure disease resistance is maintained in crops it is essential that steps be taken to guarantee a reserve of genetic variation for future breeding and to implement crop management practices which reduce the rate at which pests and pathogens can adapt to resistant varieties.

THE PROBLEM

In 1905 and 1907, Biffen reported for the first time that disease resistance in plants was inherited in a simple mendelian fashion. Since then, the investigation of the genetic basis of disease resistance has been a major objective of plant breeding because, *ceteris paribus*, resistant varieties give larger yields and an understanding of how resistance is inherited improves the design of breeding programme.

Individuals within a population vary for some characters but this variation is not all of the same type. Some characters vary continuously but others can easily be divided into two or more distinct groups. Continuously variable characters are usually controlled by many genes each contributing a small amount (polygenic inheritance) and discontinuous characters by one or few genes. Often the same character can be controlled by different genetic systems. Resistance to disease is no exception to these general principles. For example, resistance to *Helminthosporium maydis* in corn is controlled polygenically and resistance to powdery mildew (*Erysiphe graminis*) in barley is controlled by single genes. In some cases, resistance to a disease can be controlled both by polygenic systems and single genes, e.g resistance to *Puccinia sorghi* in corn. In the search for better crops all forms of genetic mechanism have been exploited by plant breeders but there is a tendency to choose simply inherited disease resistance because it is easier to handle in a breeding programme. Although the genetics of resistance is well understood, in many cases, we have virtually no knowledge at all about the control and expression of resistance.

During the 1940s Flor (1956) investigated the genetics of the flax (*Linum usitatitissimum*)-flax rust (*Melampsora lini*) system. He found that resistance in flax was controlled by

single genes with resistance dominant to susceptibility and that the ability of the fungus to infect (virulence) a given host was also controlled by single genes with virulence recessive to avirulence. He summarised his results as the "gene-for-gene" hypothesis which states that for every gene conditioning resistance in the host there is a corresponding gene conditioning virulence in the parasite. Because a small number of economically important plant disease show gene-for-gene interactions, e.g. powdery mildew of barley; stem and leaf rust of wheat, the gene-for-gene hypothesis has become a central part of thinking in plant pathology. However, only a few systems have been shown to interact in a gene-for-gene fashion and in all cases, the host plant is a cultivated crop species (Day 1974).

As crop husbandry became more scientific and processing requirements more stringent, the monoculture of crops became an economic necessity for the grower; he needed to know that his crop would react in a predictable way to his husbandry practices and his customers demanded uniformity of product. This in turn demanded genetically uniform varieties with predictable characteristics. But, large areas of uniform resistant varieties are a very efficient way of selecting rare variants capable of overcoming resistance. Once established, these variants can spread rapidly through a crop, giving rise to the "boom-and-bust" cycle characteristic of many crops under intensive cultivation, e.g. in the U.K., spring barley varieties have had an effective commercial life of only 3-4 years before increasing disease problems have led to their fall from favour with growers (Barrett 1981). The rapid "breakdown" of resistance under field conditions has left breeders trying to resolve two almost incompatible aims:

1) the recognition of the genetic vulnerability of crops and the necessity for new forms of resistance.
2) the requirement that new resistant varieties are made available rapidly.

Prior to the development of intensive cultivation, each area within a crop growing region possessed a characteristic range of land-races which were not genetically uniform. Out of this variation a range of different plant types could be selected including some plants resistant to disease; the Mlg gene which controls resistance to powdery mildew in barley was isolated from such a source (Wolfe and Shwarzbach 1978). The other principal source of new resistance is from the wild relatives of the cultivated species; the R-genes controlling resistance to late blight (Phytophthora infestans) in the cultivated potato (Solanum tuberosum) were transferred from the wild species Solanum demissum (Russell 1978). Both sources of variation are now threatened. The land-races which have provided so much material for plant breeders have now virtually disappeared from the agriculturally advanced nations. In the less developed parts of the world land-races still form the basis of present day subsistence farming and over the last half century plant breeders have systematically made collections in these countries. A "miserable-looking wheat" collected by Harlan in a

remote part of eastern Turkey in 1948 "turned out to be resistant to 4 races of stripe rust, 35 races of common bunt, ten races of dwarf bunt and to have good tolerance to flag smut and snow mould" (Harlan 1973). The advent of the "green revolution" and an emphasis on cash crop cultivation have rapidly displaced indigenous land-races from many parts of the Third World where agricultural improvement has been encouraged. For example, in 1955, Ochoa reported collecting 385 samples of previously undescribed cultivated and wild potatoes from northern Peru. At two sites, fifteen years later, he found none of the 45 indigenous varieties he had collected previously (Ochoa 1973). Agricultural progress has also had severe effects on the availability of variation from the wild relatives of cultivated plants. The pressures to bring even marginal land into cultivation has reduced the habitats of many of these plant species.

The transfer of resistance from land-races and wild relatives into an agronomically useful plant type requires many generations of backcrossing and it is many years before agriculturally useful varieties can be released. Conversely, the requirement that replacement varieties are made available rapidly means that new varieties must often be produced by crossing various permutations of currently available varieties, i.e pedigree breeding. The immediate consequence of this is that new varieties are often derived from previously successful varieties. In the NIAB recommended lists for 1982 , there are 8 spring barley varieties in the recommended and provisionally recommended categories and these varieties have 16 previously cultivated varieties in their immediate progenitors (Anon 1982). Linde-Laursen et al (1982) have shown by C-banding analysis that 10 barley varieties introduced in north-west Europe since 1970 all carry the same chromosome 3A variant which was absent from barley varieties introduced prior to 1914.

THE SOLUTION

The joint pressures of intensive crop husbandry and the narrowing of the genetic base of crops make it essential that new germ plasm be introduced but this genetic variation is rapidly disappearing (Frankel 1977: Simmonds 1962, 1979). Conservation measures are required and these can take two forms:

1) To attempt to reduce the rate of erosion of genetic resources.
2) To pursue policies which increase the long-term effectiveness of resistance.

Genetic resources.

Collections: Plant breeders have always maintained stocks of potentially useful lines and in some countries this responsibility has now been taken over by government institutions. International agencies have made great efforts to collect germ plasm for their breeding programmes. For example, the Indian national rice collection contains in excess of 20000 lines and IRRI maintains a stock of over 4000 lines from India. The way in which collections are maintained depends on the biology of

the crop species and the costs of maintaining collections will vary depending on the storage and planting facilities required (Simmonds 1979).

Gene Parks: Where areas of the native habitat of wild relatives still exist, it has been proposed that such areas be designated as "gene parks". This could permit larger populations and a wide range of variation to be maintained and also go some way to reducing the loss of variation in populations maintained in collections (Dinoor 1977).

Biotechnology: The progress made in tissue and meristem culture and pollen storage now make it feasible that in future lines of some crops could be maintained using these techniques (Simmonds 1979).

Management of Resistance.

The need for new variation could be reduced if cultivation practices were modified so that the rate of evolution of pathogens was reduced (Barrett 1981). This would extend the effective life of varieties and permit breeders to concentrate on other characters ,e.g. yield and quality, and increase the genetic base of their selections. A major cause of resistance breakdown is variety monoculture and one solution is to introduce diversity in both time and space.

Diversity in Space

Diversification: For many crops there is a range varieties each carrying different resistance genes. Planting a selection of varieties can reduce the risk of infection and thus, contribute to a slowing down of pathogen evolution on each variety. This approach requires that the genetic resistance carried by each variety is known so that varieties can be chosen on the basis of their resistance genes (Wolfe and Barrett 1977).

Multilines and Variety Mixtures: If the principle of diversification is applied to smaller and smaller fields each field eventually becomes a single plant. This is the basis of multilines and variety mixtures. In a variety mixture, varieties carrying different resistance genes are sown as a mixed stand. The population dynamics of pathogens within such a stand leads to a reduction of disease within the crop and also confronts the parasite with several adaptive problems simultaneously. A multiline strategy is in principle the same as that of a variety mixture except that the components have all been derived from a common breeding programme; apart from the resistance genes, the plants are identical (Frey et al 1977; Wolfe and Barrett 1980).

Mixed Cropping: Many crop species are effectively immune from the diseases of other crop species. By planting different species together the plants of each species are at a lower density than they would be in a pure stand and hence the rates of disease spread and pathogen evolution can be reduced. Mixed cropping is traditional form of subsistence cultivation , e.g.

bean-corn mixtures in Central America and oat-barley mixtures in north-west Europe, and recent years have seen a reappraisal of its usefulness.

Gene Deployment: Where crops are grown intensively over large continental areas there is the possibility that a serious epidemic could spread throughout the area. Gene deployment is an attempt to prevent this occurrence by dividing up the area into regions and only deploying certain resistance genes within each area (Frey et al 1977).

Crop Diversification: In areas where intensive cultivation is practised, the capital investment for each crop may effectively prevent a range of different crops being grown. If the dangers of this specialisation were more widely appreciated the growing of a wider range of crops could be encouraged.

Diversity in Time

The development of continuous cropping of a single crop species can lead to disease problems if each successive crop inherits problems created by the previous one. To reduce these risks, a return to a longer rotation of different crops could be encouraged. Where the costs of long rotation cultivation are prohibitive, some benefit can be gained by ensuring that successive crops of the same species carry different resistance genes. Multilines, variety mixtures and mixed cropping can offer a further level of diversity in such a strategy because the components can easily be varied from year to year to prevent the adaptation of pathogens to each combination of components (Wolfe and Barrett 1977).

Pure Line Approaches

Durable Resistance: Some sources of resistance have proved to be effective throughout the commercial life of varieties, e.g. resistance to yellow rust in the wheat variety Cappelle Desprez. Johnson and Law (1975) have called resistance of this type "durable resistance". Unfortunately the only way to test for durability of resistance is by exposure to disease. Consequently the more genetic variation that is available, the better the chances of detecting forms of resistance which might prove durable.

Pyramid Resistance: When the infection in each crop is not significantly affected by the crop which preceded it, levels of disease can be reduced by incorporating many resistance genes into each variety (pyramid resistance). This reduces the possibility of a pathogen assembling the right combination of virulence factors to be able to attack the variety.

These strategies present a plant pathogen with a range of problems so that adaptation to one part of the cropping system does not pre-adapt it to other parts of the system. This will reduce the rate at which a pathogen can evolve in any particular direction and, hence, reduce the disease risk.

Although the argument for the maintenance of genetic resources so far has been based on the premise that "resistance breakdown" will occur, there are many examples of genetically controlled resistance which has remained effective for many years. For example:

Today, loose smut of barley (Ustilago nuda) is a rare disease in the U.K., this has not always been the case. The exploitation of closed flowering genotypes, which prevent penetration by spores of the fungus, effectively removed this disease from the field (Russell, 1978).

Until the southern corn leaf blight epidemic of 1970, resistance to H. maydis had been gradually raised in corn cultivars in the U.S.A.. The epidemic of 1970 was not a failure of this resistance but a susceptibility to the Race T toxin conferred by Tms cytoplasm. Once Tms cytoplasm was removed from the field, control returned to its previous high levels due to the polygenic resistance (Anon 1972).

The early banana trades in the Caribbean and Central America relied on a single banana clone, Gros Michel, but between about 1900 and 1935, the area was ravaged by banana wilt Fusarium oxysporum f.sp cubense to which Gros Michel was extremely susceptible. Gros Michel was replaced by selections from the Cavendish clone, which was resistant to wilting, and this control has persisted until the present day (Simmonds, 1959).

DISCUSSION

The cropping strategies outlined above will assist the crop itself to control diseases to which it is exposed. The strategies allow a grower to spread or reduce his risk of disease loss with few or no extra inputs and the plant breeder to extend the life of his varieties and hence the financial return on his efforts. But all of these strategies require the availability of sources of disease resistance.

Most of the management methods which do not drastically alter cultivation techniques have been implemented in crops where the need has been felt at little or no cost to the grower. For the more radical measures, i.e. those aimed increasing between-crop-diversity, it is difficult to see such measures being introduced unless an overwhelmingly strong argument for economic benefit in the immediate future could be presented.

Agriculture today is viewed as an economic activity but this can limit an appreciation of genetic resistance. In natural populations there is feedback between the density of hosts and parasites; if the host population becomes too large, the parasite population increases, raising the level of infection. Crop cultivation necessarily increases the density of plant populations and so disease is an inherent part of agro-ecosystems. The existence of disease in a crop is usually seen as a problem because of its effects on yield and the variety is deemed to be "susceptible". Yet many "susceptible" varieties do show quite high levels of resistance when compared to unselected material or when tested with other parasite populations. The

classification of varieties into "susceptible" or "resistant" is determined relative to an economic baseline and not to some fundamental biological property of the plants. It is essential that these two issues are not confused. A crop variety can display high levels of disease resistance and yet be deemed "susceptible" because it displays symptoms at a level which makes pesticide treatment economic. Virtually all cultivated crops display "resistance" but in agronomic terms it is the loss of potential yield that is the criterion by which the crop is judged not the actual yield.

CONCLUSIONS

Genetically controlled resistance to disease underlies all successful crop cultivation. In some cases control of disease damage at economic levels has been sustained but in other cases it has proved ephemeral. Resistant varieties are the first level of defence against plant disease and strategies of variety use are now being considered to improve control. Even where the costs of chemical control treatments can be justified, a crop can still display high levels of resistance. To argue, naively, that resistance breeding need no longer be pursued because chemical control is available is to ignore the resistance that is already present in the crop. However, the effective use of genetically controlled resistance requires that variation is available but we are faced with an accelerating loss of this variation. Land-races and indigenous varieties are being lost and the habitats of the wild relatives of crop plants are being destroyed by agricultural and economic progress. It is essential that if crop production is to be maintained at its present levels, or increased, in the face of disease, then the genetic variability which is the stuff of both evolution and breeding should be conserved. Not only does this mean that the reserve of variability should be increased but also that the exploitation of the results of the breeders' efforts should be tempered with an appreciation of the evolutionary adaptability of pathogen and parasites. Genetically controlled resistance has been and will continue to be the most cost-effective method of disease control. But disease control demands a flexible approach. If we are not careful we may find ourselves with all the genetic defences committed and few reserves to fill the breaches.

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BREEDING STRATEGIES FOR MILDEW AND SCAB RESISTANCE IN APPLE

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Background and objectives

Mildew (Podosphaera leucotricha) and scab (Venturia inaequalis) resistant apple varieties are being developed in order to reduce pesticide spray applications and production costs and to satisfy a growing consumer demand for fresh produce grown under minimum spray regimes. Resistance in present commercial varieties is commonly under polygenic control and rarely sufficient to permit a significant relaxation of the spray programme. Attempts to incorporate such low levels of resistance can drastically handicap breeding progress in complex breeding programmes aimed primarily at combining high yield and high quality.

High levels of simply inherited resistance, sufficient to allow a complete relaxation of chemical control, have been found amongst small-fruited Malus species and introduced into a commercial breeding programme. Breeding and selection procedures are required to provide a rapid improvement in fruit size combined with high yield and good fruit quality, while providing maximum security against any breakdown of host plant resistance.

Materials and methods

Malus robusta (MAL 59/1) and M. zumi (MAL 68/1) provide reliable sources of mildew resistance maintained for over 17 years in unsprayed plots at East Malling and successfully tested at other sites in Europe and N. America. Resistance is determined by two dominant genes in each species. Of the five genes known to provide resistance to all known races of V. inaequalis the most widely used is V_f from M. floribunda. Resistant varieties carrying V_f are available.

Resistance screening is done in a glasshouse five weeks after germination for mildew and six weeks for scab following spray inoculation at the two leaf stage. Effective screening for mildew resistance can also be done 11 weeks after germination, following inoculation at the seven leaf stage. Further observations are made in the field 18 months after germination prior to budding resistant selections on to M.27 rootstocks.

Results and conclusions

The high levels of resistance provided by the major genes are enhanced in combination with polygenes for resistance. This is achieved by using commercial varieties with moderate resistance in the crossing programme.

Glasshouse selection is successful for both diseases. The proportion of scab free seedlings varies according to the polygenic component of the parents. Most M. zumi and M. robusta mildew resistant derivatives show some sporulation; resistant seedlings are selected by grading sporulation. In the field M. zumi resistant seedlings show no symptoms but M. robusta resistant derivatives show varying degrees of necrosis on the underside of leaves occasionally accompanied by minute sporulating areas in the axils of the veins. The degree of leaf necrosis is related to the degree of polygenic resistance. The deliberate selection of seedlings combining major genes with polygenes is a means of safeguarding the crop against new gene specific races of the pathogens. Resistance is regarded as a bonus, the aim is to produce new varieties with improved features for which they will be maintained in cultivation whatever the response to mildew or scab.

The use of large fruited culinary apples as parents in the early generations enabled commercial fruit size to be reached by the second backcross. High yield potential has also been derived from the Malus species used in breeding for scab and mildew resistance by selecting precocious seedlings fruiting only four years after germination, which also carry full resistance to the two diseases.

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5B—R2

THE BEHAVIOUR OF SUGARCANE VARIETIES TO SMUT INFECTION IN KENYA

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Background and objectives

Ustilago scitaminea, the fungus responsible for culminocolous smut in sugarcane in many parts of the world is one of the most serious diseases in Kenya. To curb its spread, a number of control measures are recommended and include the growing of resistant varieties as the most satisfactory long term control measure. This involves identification of resistant genotypes through smut screening trials before recommending them. The objective of the research reported herein was initiated to study the behaviour of 25 sugarcane varieties to smut in relation to the stools and stalks infected in the field.

Materials and Methods

The study was conducted in the plant, first and second ratoon crops at National Sugar Research Station, Kibos. Introduced varieties CB38-22, CB45-155, Co331,Co419, Co421, CP56-20, CP56-39 and 18 locally bred varieties (with EAK numbers) were used in the studies. Healthy three budded setts were inoculated by immersing them in freshly collected smut spore suspension. Twenty setts per plot were used in a randomized block design with four replications. Stools and tillers infected were determined by monthly counts up to 9 months after planting or harvesting.

Results and conclusions

Percent smut stools was calculated and assigned numerical ratings ranging from 0-immune to 9-very highly susceptible. Varieties with a value of 5 and less are acceptable while those with more are not. The behaviour of the varieties was determined by studying the pattern of stool and tiller infections in the three crop cycles and categorized into four groups.

The main characteristics of the first group were the reactions which ranged between highly resistant to intermediate throughout the assessment period. There was very little increase or decrease in the stools or tillers infected between crop cycles. Comparatively there was a higher % of stool infected than whips produced. Varieties EAK71-293, EAK71-183, CB38-22, EAK71-193, Co421, Co331, EAK71-402 and EAK71-496 fall into this group. Popular varieties Co421 and Co331 fit in this pattern of smut development and this behaviour may explain why they have been in commercial production for over 20 years with little annual variation in smut reaction.

The second group in which varieties CP56-20, CB45-155, EAK71-476, EAK71-526, EAK70-39, EAK70-153 and N55-805 were categorized had acceptable reactions in the plant crop but unacceptable in the ratoons. The highest variety mean stools and tillers infected were recorded in the first ratoon.

Group three had varieties with a large number of smutted stools in the plant crop and a drop in the ratoons. Varieties EAK70-30, EAK70-27, CP56-39 and EAK71-242 were categorized into this group.

Varieties in group four had very high smut infections in the plant crop and continued through the ratoons. Very highly susceptible varieties as EAK70-16, EAK70-150, Co419, EAK71-508, EAK71-357 and EAK70-156 had this pattern of smut development. The highest infection occurred in the first ratoon. (Whittle and Walker 1982) reported similar results.

Proportionally higher % of stools were infected than whips produced in all the groups. Since varieties differ in stalk numbers per unit area, the use of stalks infected to determine variety resistance may underestimate the resistance. The use of stools to rate varieties is a logical measure of resistance as the number of stools per unit area does not differ greatly from variety to variety.

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PATHOGENIC VARIATION IN ASCOCHYTA RABIEI

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Background and objectives

Ascochyta blight is a severe limitation to chickpea (Cicer arietinum) production in the Mediterranean region and the Indian sub continent. The disease is particularly serious in cool wet weather. This discourages winter sowing in some countries with a consequent loss of yield potential as a spring sown crop grows on residual soil moisture. Breeding strategies at the International Centre for Agricultural Research in Dry Areas (ICARDA) include the incorporation of resistance to A. rabiei in the chickpea improvement programme. Some cultivars with resistance to A. rabiei have not maintained it at different locations over different seasons. The objective of this work is to study the pathogenic variation of isolates of A. rabiei sent to Reading by collaborators from throughout the chickpea growing regions.

Materials and Methods

Seedlings of a selection of cultivars provided by ICARDA were grown in propagation trays. When 10-15cm tall the plants were sprayed until run-off with spore suspensions ($40 \times 10^4 ml^{-1}$) prepared from 6 day old cultures. After inoculation high relative humidity was maintained for 4 days by covering the trays with transparent lids. Temperatures during this period were maintained at 20°C. Disease assessments were made 21 or more days after inoculation using a 1-9 scale of infection based on the extent of lesion development on leaves and stems, stem die-back and breakage.

Results and conclusions

One isolate (Sy2) supplied by ICARDA from the Ascochyta disease nursery at Tel Hadya, Syria appears more pathogenic than other isolates from Syria, Lebanon, Tunisia, Morocco, Spain, Pakistan and India. In some experiments this isolate was lethal to all cultivars tested. Five kabuli type chickpea cultivars from ICARDA (ILC-72, -191, -194, -202 and -3279) which were among those previously recorded as resistant in Ascochyta disease nurseries in Syria, Lebanon, Algeria, Turkey and Pakistan showed resistance or moderate resistance to all isolates already mentioned except two from Syria. Other results have been less conclusive and some experiments need repeating, however, there is as yet no clear evidence that the fungus shows differential pathogenicity on the cultivars tested.

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5B—R4

PATHOGENIC VARIATION OF PEARL MILLET DOWNY MILDEW

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Background and Objectives

Pearl millet (Pennisetum americanum (L.) Leeke) is a staple food crop of the semi-arid tropics, particularly West Africa and India. Downy mildew caused by the oomycete fungus Sclerospora graminicola is the most damaging disease of pearl millet, particularly on high yielding hybrid cultivars. Both crop and pathogen are outbreeding which means that the pathosystem is potentially highly adaptable. Breeding for resistance has had some success but it has been necessary to devise means of assessing variability in the system to estimate the risks associated with particular cultivars. In international nurseries certain cultivars displayed more downy mildew resistance in some locations than in others; the cause could have been environmental or based on host-pathogen genotype interactions and experiments were set up at Reading to investigate this problem.

Materials and Methods

Oospores in dried leaf material were collected from various locations in West Africa and India. Asexual inoculum was multiplied through a susceptible cultivar in polythene tunnels and a range of cultivars were exposed to zoospore in tunnels and in isolation plant propagators. Disease was assessed as percentage incidence of symptoms.

Results and Conclusions

Disease incidence was generally greater from West African isolates than from the Indian isolates and these reuslts confirmed the indications from the international trials. The Finlay/Wilkinson regression model for the study of genotype-environment interactions was modified using the mean incidence of the isolates over all cultivars as a measure of their pathogenicity. The components of variance were separated according to continent of origin of both host and pathogen. Most cultivars had "average" stability, disease incidence increased steadily in response to increased pathogenicity. Some cultivars showed "phenotypic stability" to Indian isolates only and disease increased only slightly with increase in pathogenicity. An ideal cultivar would show phenotypic stability across all isolates. Some cultivars were unstable and disease incidence rose steeply with only small increases in pathogenicity.

Cultivars from West Africa were more unstable in respect of Indian pathogens in spite of the lower actual levels of pathogenicity; conversely Indian cultivars were more susceptible to West African isolates. These observations enhance the evidence for differences between the pathogen populations on the two continents. It is clear that resistant host material should be bred under exposure to the indigenous pathogen population. Cultivars which are phenotypically stable should be suitable for use, at least in the short term. The relationship between this stability and durability of the resistance has yet to be investigated.

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DEVELOPING BARLEY VARIETIES WITH PROTECTED RESISTANCE TO BROWN (LEAF) RUST.

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Background and objectives

In the barley brown rust system, a number of major Pa genes for resistance have been identified and numbered 1 - n. This resistance is designated Type 1. Another type of resistance, designated Type II, is expressed through the slower development (increased latent period) of fewer, smaller pustules (decreased infection frequency) resulting in a low rate of disease increase in the field, the slow rusting effect. To minimise the effects of the "breakdown" of Type I resistance, it is desirable to evaluate the level of Type II resistance in the presence of effective Type I resistance and more positively, to develop breeding techniques for combining both resistances into the same cultivar.

Experimental

Certain characteristics of some Pa genes offer the possibility of assembling more than one into a single genotype and of combining them with slow rusting resistance. The genes of most value in this respect are Pa_3 and Pa_9 to which virulences are rare and gene Pa_7 to which virulence has not been detected in Britain.

Controlled climate studies involving carriers of these genes, namely Estate (Pa_3), Cebada Capa (Pa_7) and C.I. 1243) (Pa_9) have indicated that the action of Pa_7 and Pa_9 is temperature sensitive and resistance is not expressed below 5°C in the former case and above 20° in the latter and this offers several possibilities for manipulating these genes in segregating populations.

Single temperature sensitive resistances can be combined with slow rusting resistance by screening against normally avirulent isolates at appropriate temperatures. For example, gene Pa_7 or Pa_9 can be combined with slow rusting resistance by first screening the F_2 population with an avirulent isolate at a temperature at which the major gene resistance is expressed. The selected resistant plants are then inoculated quantitatively with an avirulent isolate and screened at a temperature at which the major gene is inoperative. The selected plants will therefore carry the major Pa gene in a slow rusting background. Such resistances need to be effective over the temperature range normally encountered during active disease development in the field as are genes Pa_7 and Pa_9. An additional scheme which does not suffer from these limitations can be postulated from the results of other studies.

Some Pa genes allow penetration and colonisation by avirulent strains of the pathogen to the stage at which sporulation would normally occur. At this point rapid death of host cells occurs and sporulation is prevented. The visible expression of this interaction is the occurrence on the leaf of pin-head sized necrotic sites. Following uniform inoculation, there is a correspondence between the number of necrotic sites elicited by an avirulent strain on cultivars carrying gene Pa_2 (Batna, Peruvian and Ricardo) and the number of pustules produced on these cultivars by a virulent isolate. The number of necrotic sites is governed by and is an expression of, the level of Type II resistance of the cultivar (Clifford, 1974). A comparative histological study of cv. Vada (Type II resistant) and the Pa carrier Cabada Capa (Clifford and Roderick, 1981) demonstrated that up to the time of sporulation, penetration and colonisation in Cebada Capa proceeded in a way similar to that in Vada. It is concluded that the slow colonisation in Cebada Capa is governed by a resistance mechanism similar to that in Vada. The selection of segregants combining such effective major genes with Type II resistance is simple; plants with low number of infection sites being selected after uniform inoculation with an avirulent pathogen strain. The large numbers of plants in a segregating population precludes a detailed histological assessment although this can be carried out subsequently on the few potential cultivars selected from a cross.

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5B—R6

FUNGICIDE INTEGRATED INTO HOST MIXTURES FOR DISEASE CONTROL

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Background and objectives

Inbred disease resistance can be cheap and effective, but it may lack durability and it may not totally eliminate economic damage. Thus, the supplementary use of fungicides is often favoured in high input systems with appropriate pricing structures.

Unfortunately, fungicides may be subject to the same drawbacks as resistant varieties. Consequently, attempts have been made to define and use both resistant varieties and fungicides that are inherently durable. However, the system in which both are used can also be designed to provide a high degree of control and improved durability.

Results and discussion

By using limited fungicide treatments on relatively resistant varieties, disease control can be maximised, and the evolutionary response of the pathogen retarded.

A more sophisticated approach is to integrate the use of a fungicide treatment into a variety mixture strategy. For example, in a mixture of three barley varieties, a mildew fungicide can be applied to the seed of only one component. This can be changed to other components in subsequent years, or the components can be changed. The advantages are, first, that the combination of resistant varieties and fungicide provides a high level of disease control. Second, the cost of fungicide application is reduced to one-third of that of a conventional treatment. Third, selection on the pathogen is highly diversified which will slow down the rate of pathogen response relative to that on a single variety or fungicide.

For example, pure stands of Atem, Carnival and Triumph were grown untreated, or from seed treated with triadimenol at the commercial rate. These treatments were compared with different methods of applying one-third of the normal dose. Since there was no apparent difference between the latter methods, the data for the one-third treatments were combined in Table 1. The same treatments were applied to a mixture of the three varieties, but in addition, mixtures were grown in which one of the varietal components was treated with the normal dose and then mixed with untreated seed of the other two components.

Table 1. Mean yield (t/ha^{-1}) of Atem, Carnival and Triumph barley, and of their mixture, untreated or treated with triadimenol at one-third, or at the commercial rate (lsd $P < 0.05$: within rows 0.03, between rows, 0.26).

	Untreated	1/3 Fungicide	1/1 Fungicide
Mean of 3 vars.	8.33	8.65	8.84
Mixture	8.70	8.86*	9.10

* mean yield of mixtures with one component treated was 9.04 t/ha^{-1}, sig. more ($P < 0.05$) than mean of three varieties at 1/3 fungicide treatment.

From Table 1, overall, mixtures yielded more than pure stands ($P < 0.05$). Fungicide treatment increased yield but this was significant ($P < 0.05$) only for the pure stands. More important, although the normal treatment tended to outyield the one-third treatment, the difference was not significant. Further, the yield of mixtures with single components treated was significantly greater ($P < 0.05$) than that of the pure stands with the same treatment. It therefore appears that to maximise yield and minimise cost, the best strategy appears to be application of fungicide to a single variety in the mixture.

Maris Tricorn

To put this strategy into practise, the Plant Breeding Institute and the National Seed Development Organisation are developing a mixture, Maris Tricorn, which has the three varietal components, Carnival, Porter and Tasman. These varieties are marginally higher yielding and have malting quality similar to or better than Triumph, but differ in their mildew resistance genotypes. In ten trials without fungicide treatment in 1982, the mixture yielded as well as the best component, Tasman. In three trials in which all components and the mixture received two fungicide treatments, the mixture ranked second to Porter in yield.

In trials of Maris Tricorn in 1983, Porter has been treated with a triazole fungicide. The reasons for treating Porter were, first, because it has the largest fungicide response of the three components. Second, although Porter has good adult plant resistance, it shows some susceptibility in the early growth stages, when a seed treatment is more effective than variety mixing. Third, it would be imprudent to apply a triazole fungicide to Carnival, since recent surveys have shown that there is an association between insensitivity to the triazoles and pathogenicity for varieties with the same resistance as Carnival.

METABOLIC PROFILING AS A POTENTIAL AID TO BLACK CURRANT BREEDING

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Background and objectives

Metabolic profiling is a technique in which samples are analysed chemically and possible correlations between the presence and concentration of individual or grouped components and the biological status of each sample are identified. This approach to screening has been adopted in an attempt to reduce the lengthy procedures presently involved in black currant breeding.

Two serious problems in the cultivation of black currant (Ribes nigrum) are American gooseberry mildew (Sphaerotheca mors-uvae) and gall mite (Cecidophyopsis ribis), vector of reversion virus. In a continuing breeding programme, the genes Sph_2 (from Scandinavian black currant), Sph_3 (from Ribes glutinosum) and Ce (from the gooseberry) are providing strong resistance. Up to 7,000 seedlings are screened annually. Screening for Sph_2 or Sph_3-based resistance to mildew, but not Ce-based resistance to gall mite, is easily done in the glasshouse. Identifying Ce-carrying plants in their first year would save 3-4 years' field screening and economise on land and labour.

Materials, methods and results

In a pilot study, leaves from young black currant selections, grown from seed under glasshouse conditions, were extracted and the extracts analysed by programmed-temperature gc-ms. The mass spectrometer was used as a detector specifically to allow for the later identification of any components shown to be significantly correlated with resistance or susceptibility. The total-ion-current profiles provided information on the occurrence and relative abundance of the volatile components of each extract.

From the total data set, those components which occurred in 50% or more of the samples were selected. This reduced data set, combined with subsequent information on the field response of each selection to gall mite and/or mildew, was studied by multivariate statistical techniques (see Ref.). Principal components analysis was used to distinguish 'outliers' in each of two families of selections. On weighting the peaks, by canonical variates analysis, to maximise the differences between resistant and susceptible selections, the apparent success rate in prediction became 100% and 95% for mildew in the two families. Stepwise discriminant analysis provided information on the stability of the discriminations and a more realistic estimate of the likely success rates.

In a second pilot study, using superior chromatographic and data capture equipment, samples from the same families provided criteria of resistance. Two other families, not fully characterised for field response, were analysed and the criteria of resistance applied to their peak profiles in a test of the predictive power of the metabolic profiling technique.

Conclusions

While the present results are encouraging, the criteria of resistance require confirmation from larger data sets. The components which are significant markers of resistance will be identified; one or more may be biologically active against gall mite or mildew. If proven successful, the technique should be transferred to conventional glc equipment to allow for the analysis of very large numbers of selections. The technique should be applicable to many other host-pest or host-pathogen interactions.

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5B–R8

THE USE OF GENETIC RESISTANCE IN TOMATO FOR THE CONTROL OF TOMATO MOSAIC VIRUS

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Background and objectives

Tomato Mosaic Virus (TMV) is an important pathogen of the glasshouse tomato, Lycopersicon esculentum Mill., and may cause up to 25% loss of economic yield (Broadbent, 1964). Control of this disease is achieved mainly by the use of resistant varieties possessing one or more of the TMV resistant factors Tm-1, Tm-2 or Tm-2^2. The development of virus strains 1 and 2 with the ability to overcome Tm-1 and Tm-2, respectively, has reduced the effectiveness of these genes. In consequence extensive reliance is now placed upon the Tm-2^2 factor in tomato production in N.W. Europe. Despite this widespread usage there has been little evidence of a new strain (strain 2^2) establishing which can overcome the Tm-2^2 gene but under certain conditions, in particular high temperatures and high virus inoculum levels, invasion by strain 0 may induce a hypersensitive resistance reaction (systemic necrosis) which reduces yields dramatically (Hall, 1980). Experiments were conducted to test the relative durability of the three resistance genes and also the virulence of newly established virus strains.

Materials and Methods

A series of isogenic tomato lines based on cv. Craigella, differing only in their TMV resistance (Pelham, 1968) was used to create grafted plants comprising susceptible stocks and scions possessing either Tm-1, Tm-2 or Tm-2^2. The scions were inoculated through the graft from a reservoir of strain 0 in the infected stock. This method subjected the virus to high selection pressures. It was intended by using small numbers of grafted plants to simulate the use of resistant varieties in large scale commercial cultivation. Samples were taken from the scions at intervals over a 20 week period and tested for presence of virus and for strain type by contact inoculation to seedlings of the differential series.

Results and conclusions

Twenty weeks after graft inoculation with strain 0, 76% of Tm-1/+ scions yielded strain 1, 80% of Tm-2/+ scions yielded strain 2 and 54% of Tm-1/+, Tm-2/+ scions yielded strain 1.2. indicating that these two resistance genes had been overcome. Within three to six weeks of inoculation Tm-2/+ scions developed systemic necrosis, but at this stage only low concentrations of strain 0 could be detected. From eight weeks typical mosaic symptoms usually developed, and strain 2 could then be recovered from the scion. Homozygous Tm-1 and Tm-2 conferred significantly higher levels of resistance in terms of rate and frequency of new strain establishment than the respective heterozygous forms. Strain 2^2 types were not detected in 212 Tm-2^2/+ or 234 Tm-2^2/Tm-2^2 scions, but systemic necrosis frequently occurred in both genotypes (more so in Tm-2^2/+). Further tests revealed virus titres to be about 10% of those in plants showing typical susceptible reactions, and that isolates had strain 0 characteristics, although as a result of host passage some had acquired greater capacity for inducing necrosis in contact-inoculated Tm-2^2/+ test plants. These results suggest that it would be feasible for similar necrosis-causing types to develop under commercial conditions, especially when Tm-2^2/Tm-2^2 cultivars are grown in infected border soils at high temperatures. It would be particularly serious if virus types capable of inducing a high frequency of systemic necrosis in plants at normal temperatures became established. In such cases it may be advisable to use two or three resistance genes in combination. Widespread losses from this disorder are less likely to occur in crops grown isolated from the soil e.g. in peat modules where there is considerably less endemic TMV inoculum; in these circumstances effective protection should be given by Tm-2^2/Tm-2^2 genotypes. The potential danger from extensive systemic necrosis could be further minimized if breeders were to utilize existing variation and develop cultivars with low a propensity for producing this response.

When Tm-2 is considered there appears to be a link between necrosis and the development of strain 2. In view of the allelism of Tm-2 and Tm-2^2 it is possible that a similar association occurs with Tm-2^2. Those isolates which caused increased necrosis in Tm-2^2/+ plants may have been precursors of new aggressive 2^2 strains. Even so, the work reported here indicates that further changes in virus pathogenicitiy and the development of aggressive isolates of strain 2^2 are unlikely to be frequent.

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COMPARISON OF VIRULENCE OF XANTHOMONAS CAMPESTRIS PV. ORYZAE IN SOUTH CHINA AND IN PHILIPPINES.

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Background and objectives

Rice bacterial blight, caused by Xanthomonas campestris pv. oryzae has become more severe in recent years in China. The need for the development of resistant varieties offers to be the most promising means for reducing the incidence of bacterial blight. For many years, physiologic specialization and pathotypes of X. campestris pv. oryzae have not been well understood. Controversial results were reported from Japan, IRRI and other Asian countries. However, variation in virulence from one country to another, and also from one locality to another in the same country was generally recognized. In order to clarify the situation of how variable is pv. oryzae in terms of their virulence in South China and in Philippines, IRRI and CAAS Guangdong Branch initiated collaborative research on a such project in 1980-1981. This paper reports on a study in comparing the differential interactions of Japanese, IRRI and Chinese differential varieties with Chinese and Philippine pathotypes.

Materials and Methods

Four Philippine races namely race I,II,III,IV and five Chinese pathotypes namely groups I,II,III,IV and V were evaluated for their virulence on thirteen Japanese, IRRI and Chinese differentials. Rice seedlings were transplanted individually to pots and were grown in a greenhouse. Bacteria were cultured individually on Wakimoto's potato semi-synthetic agar medium and incubated at 28°C. The 48-hr-old cultures were used to prepare the inoculum adjusted to a concentration of 10^{8}-10^{9} cells/ml. Rice plants were inoculated at 50 days after sowing by clipping method. Ten to fifteen leaves per plant were inoculated for each variety and isolate combination. Disease reactions were assessed at 15 days after inoculation according to IRRI's Standard Evaluation Systems for rice based on lesion area over leaf area.

Results and conclusions

Comparison of virulence of the isolates in South China (Guangdong) and in the Philippines on the same set of International differential hosts revealed that the pathotype groups III and IV of South China correspond to the Philippine race I, and the group V corresponds to the Philippines race IV. About 100 out of 211 isolates (70.61%) of pv. oryzae belong to groups III and IV. Therefore, groups III and IV which appear widely distributed in South China and serve as the predominant pathotypes in Guangdong China were not virulent to the resistant variety IR 26. IR 26 was susceptible to bacterial blight in certain districts in the Southern part of the province which might be due to the presence of group V isolate virulent to that variety.

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5B—R10

SELF-DEFENCE CHEMICALS OF PLANTS

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Background and objectives

Genetic resistance is of significance to plant protectionists in several contexts. These include the development of resistance to insecticides by insects or to herbicides by weeds; the varying resistance of pest organisms to agents of biological control; the production of "biological herbicides"; and the exploitation of disease resistance in some crop plants, an example of a self-defence capability. Self-defence has been associated with the secondary chemical compounds which are omnipresent in plants. The available data suggest that weeds are better able to defend themselves by means of secondary chemical compounds than are conventionally useful agricultural species. Levels of secondary compounds in crop and pasture plants are usually lower than in weed members of the same botanical family and may be too low to be effective in self-defence. They have, however, proved to be genetically manipulable and current plant breeding strategies might usefully be amended to more actively pursue a self-defence capability.

Experimental

Mintweed, *Salvia reflexa* (Lamiaceae), is being studied under controlled and field conditions. Both foliage vapours and foliage washings adversely affect germination and seedling growth of test crop species. GCMS analyses have indicated the presence of several monoterpenes, including α-pinene. An additional, as yet unidentified, toxic non-terpene component has been recovered during HPLC analyses. Secondary manifestions, for example, reduced length of seminal root in test species (Table 1) reflect subtle, primary effects on seedling metabolism which resemble modes of action currently sought in herbicides.

TABLE 1

Length of longest seminal root of wheat seedlings 3 days after imbibition under controlled conditions (means of four replicates each of twenty seeds).

	Control (distilled water)	Ether extracted foliage washings*	Aqueous foliage washings	L.S.D.
Length of seminal root (mm)	70.4	58.6	46.3	8.5 (5%) 12.2 (1%)

*monoterpenes removed

It has been established that the magnitude of any toxic effect of mintweed secondary chemicals is modified by soil type, soils of relative high clay content tending to ameliorate phytotoxicity.

Discussion

α-pinene is here identified with allelopathy, biochemical interactions between plants, which may be a part of plant self-defence. In recent research reports α-pinene has also been associated with the defence of pine trees against weevils (Finland); allelopathy by shrubs against herbs in chaparral communities (California); deterrence of grazing by mule deer (Canada) and, in association with Douglas fir, inhibiting the growth of a variety of bacteria and a yeast (Washington/Oregon, U.S.A.).

That terpenes are widely distributed secondary chemical compounds in plants is well-known but their apparent occurrence as agents of self-defence in a range of relationships between plants and other organisms appears not to have been previously recognised. Most examples here cited are in non-agricultural associations but, given the presence of various types of secondary compound in agricultural plants, the exploitation of the defensive potential of these chemicals offers an approach complementary to the continuing use of synthetic chemicals in plant protection.

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THE ROLE OF TRYPSIN INHIBITORS IN CONFERRING BRUCHID RESISTANCE IN COWPEA

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Background and objectives

The Cowpea is one of the principal grain legumes of West Africa and north-east of South America where it provides a major source of dietary protein. Loss during storage due to the bruchid beetle Callosobruchus maculatus is widespread with up to 100% seed damage after 5 months storage. Not only is total weight loss of seed important, but also the proportion of seeds damaged, since this not only affects palatability and food value, but also the germination potential. The International Institute of Tropical Agriculture (IITA) in Nigeria initiated a breeding programme to select for resistance against this pest, and out of 5000 accessions only one (variety TVu 2027) showed significant seed resistance towards the larvae of C.maculatus, both in terms of percent adult survival and developmental time. Previous studies showed that resistance was not due to physical factors, but to various biochemical factors, in particular the elevated levels of trypsin inhibitor found in the resistant variety relative to susceptible varieties. The objective of this study was to develop a suitable bio-assay whereby development and subsequent behaviour of the insect paralleled those on the host plant and then test the toxicity of the purified cowpea trypsin inhibitor.

Materials and Methods

A culture of C.maculatus from Campinas, Brazil, was reared and maintained in controlled temperature humidity chambers (70% r.h.,27°C). The effects of the trypsin inhibitor and other potential antimetabolites were investigated by allowing C. maculatus larvae to develop on pellets of meal of susceptible varieties to which the various components had been added. The pellets were prepared by adding a calculated volume of distilled water (control), or a solution containing the fraction to be bio-assayed to give a final known concentration, to the seed meal so as to form a thick paste which was thoroughly mixed so ensuring uniform distribution of added components. The paste was then formed into pellets of approximately 0.5g, dried for 24h over silica, re-equilibrated at 27°C, 70 r.h., weighed and covered with 'clingfilm' PCV sheeting. Each pellet was placed in individual 5 x 2.5 cm glass vials closed by a filter paper stopper, and infested with C.maculatus adults; subsequently these adults were removed. Forty one days after initial oviposition the pellets were dissected and examined and the results expressed as number of adults/gram of diet.

The trypsin inhibitor from cowpea seeds was purified from the meal by trypsin affinity column chromatography.

Results and discussion

The developmental time of bruchids on the pellets, i.e. from egg hatching to subsequent adult emergence, was very similar to that on the seed. There were also no detectable morphological or behavioural differences compared to adults reared on seeds. These pellets therefore provide a favourable bio-assay whereby the effects of potential antimetabolites on larval development can be meaningfully monitored. The standard error in number of adults/g diet for the number of replicas used for each treatment was ± 10%, or less.

When affinity purified cowpea trypsin inhibitor (CPTI) was incorporated in to the diet at levels comparable to those present in the resistant variety, adult emergence was significantly reduced demonstrating that this inhibitor is an effective antimetabolite of C.maculatus. Developmental time from oviposition to adult emergence is also an important criterion in measuring the degree of resistance as this influences the rate at which a bruchid population builds up and becomes established. Adult emergence was found to take about 20% longer on pellets containing the higher levels of CPTI compared to the controls. Thus the results clearly demonstrate that addition of CPTI to a susceptible variety conferred resistance and that this effect was additive. Trypsin inhibitors from other legumes, e.g. soyabean and limabean were significantly less antimetabolic towards C.maculatus.

Thus the bio-assay described above provides a means of assessing the toxicity of potential antimetabolites and in cowpea provides convincing evidence that the CPTI is responsible, at least in part, in conferring 'field' resistance in variety TVu 2027.

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5B—R12

SCREENING STRATEGIES FOR RESISTANCE OF SORGHUM TO CHILO PARTELLUS

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Background and objectives

The sorghum stem borer, Chilo partellus, can cause substantial crop losses due mainly to deadheart formation in infested plants and thus it is important to identify sources of resistance to this insect.

C. partellus adults often lay their eggs on leaves close to the base of the plant. The larvae then climb to the whorl, where they feed. Older larvae leave the whorl and bore into the stem. The greatest mortality of larvae occurs in the initial climbing phase, suggesting that a successful climb is critical for larval survival and that a resistance to C. partellus could result simply from failure to reach the feeding site. We have examined the climbing behaviour and establishment of C. partellus on two cultivars of sorghum, with the aim of providing information of use in the improvement of screening techniques and also of identifying mechanisms of resistance. This work has been done in cooperation with the International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Hyderabad, India.

Materials and methods

Two sorghum cultivars, IS1151 and IS2205, were planted in three successive rainy seasons at ICRISAT. In establishment experiments, egg masses of known number of eggs from a culture reared on artifical diet were attached individually to leaves near the base of the plants. Plants were infested at two ages, 25-40cm and 100-160cm in height, and destructively sampled at 6h, 1,3,6,15 and 22 days after hatching. Surrounding plants were also sampled on each occasion to measure dispersal. For behavioural observations, test plants of both cultivars were selected at the above stages of growth. Observations were made using newly hatched larvae by placing an individual on the test plant in the field at the level of the lowest leaf. Its behaviour was recorded continuously until it reached the whorl, settled elsewhere, or left the plant.

Results and conclusions

Observations indicated that many larvae failed to reach the whorl, more being successful on young plants of IS1151 than on IS2205, and climbing was faster on IS1151. The effect was reversed on older plants with more insects reaching the whorl on IS2205.

In establishment experiments, sampling of plants 6h after hatching showed significantly more larvae were established in young plants of IS1151 than IS2205. The trend was reversed for older plants. Later samples showed a large fall in the number of larvae in the whorls after 24h, a further decline in the three day sample, and thereafter a stabilisation. The initial cultivar difference was maintained up to the final sample at 22 days by which time many larvae were pupating.

More surrounding plants of IS1151 were infested by dispersal from the young test plants than of IS2205, while the converse was true for older plants.

We conclude that the initial establishment of larvae in these two cultivars is important in determining the overall survival, and establishment is determined by the relative success of the larvae in reaching the whorl. The implications are 1) that screening methods should include this phase of larval life, since if it is omitted (e.g. when larvae are dispensed directly into plant whorls), critical sources of resistance may be lost; 2) that if factors influencing the climbing success of larvae can be identified these may be incorporated into a breeding programme, and simple screening methods may be developed and 3) since susceptibility changes radically with age it is important to decide the age of plant at which resistance will be most beneficial.

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RESISTANCE IN COTTON TO WHITEFLY (Bemisia tabaci)

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Background and objectives

In recent years, the cotton whitefly (WF) has caused severe damage to cotton in many countries by reducing yield, quality and marketability. In the Sudan Gezira, the WF accounts for 2/3 of plant-protection costs and the present methods of control are unsatisfactory. This paper reports the results of screening cotton germplasm for WF resistance and of an experiment to identify and quantify the characters conferring resistance together with the mechanisms involved.

Materials and methods

The Shambat Type Collection, local cv, exotic germplasm/cv and near-isogenic lines differing only in leaf-hair density (LHD) were screened in the field by periodically counting WF adults and scales and grading the intensity of WF honeydew on a 0-5 scale. Adults were also counted on lines differing in gossypol (G) levels and leaf shapes (LS): normal (NL), okra (OL) and super okra (SOL). Further, the effects of 3 LHDs: low (L) 12-46, medium (M) 51-123 and high (H) 243-342 trichomes/cm^2; and 3 LSs (quantified as NL: 0.58-0.59, OL: 0.31-0.42, SOL: 0.20-0.26; based on LS index = leaf area / leaf length x leaf width) on WF were examined in 1982 in a 3x3 factorial experiment of randomized complete-block design in a checkerboard layout. On 5 occasions, WF adults were counted in the field and WF scales, pupae and parasitized pupae were counted microscopically in the lab using standard methods. Leaf area was measured with a LI-3000 Portable Area-Meter. Microclimate was studied within Barac (67)B (NL, MLHD commercial Acala cv) and Sudac-K (SOL, LLHD Acala line) canopies. Dry and wet-bulb readings were taken with thermisters placed 23cm above the soil in the centre of 12x8m plots. Temp were recorded every 15 min over a 120-h period in Dec. and r.h. was calculated. Insecticide sprays were not applied. Appropriate statistical analyses were performed.

Results and conclusions

In 80/81, Okra 'I' (SOL,LLHD germplasm) carried considerably fewer WF adults per leaf than 15 NL germplasm/cv. During both 81/82 and 82/83, Gossypium arboreum bengalensis and G. hirsutum palmeri showed WF resistance as did 14 of the 26 OL/SOL lines tested (13 hirsutum and 1 G. barbadense viz. Pima Okra). None of the 64 NL cottons (51 hirsutum and 13 barbadense) showed adequate WF resistance.

In 81/82, HG-6-1-N (high G line) had high WF numbers. In 82/83, among pairs of near-isogenic and similar G-level lines, those with LLHD had fewer WF than those with HLHD, but G-level did not influence WF numbers.

In the factorial experiment, LLHD and/or deeply-lobed cotton leaves (OL and SOL) significantly reduced the number of WF adults/leaf and adults, scales and pupae/unit area. WF adults/leaf on the most resistant line (ORS-13: LLHD,OL exotic hirsutum cv) did not exceed the economic threshold level (200 WF/100 leaves) throughout the season. There were significant positive correlations between LHD and number of WF adults/leaf and adults, scales and pupae/unit area. LLHD conferred greater resistance than OL or SOL, the two traits accounting for 40% and 20% respectively, of the total variability in the data. The rate of increase of WF numbers over the season on susceptible lines was greater than that on resistant lines. Further, scale parasitization was 30% higher on the OL,LLHD line. Host searching and attack by a whitefly parasite is known to be more successful on smooth than on hairy foliage.

The LS influenced the microclimate. Both day and night r.h. were considerably higher (54 and 95% r.h.) in NL than in SOL canopy (43 and 76% r.h.), but the temperatures were slightly lower (27.9 and 14.7°C) in the former than in the latter (29.1 and 15.1°C). Lower r.h. and higher temp. is known to affect WF development and survival.

The marked reduction (62-92%) in WF numbers on Sudac-K over that on Barac (67) B in different experiments, may have been due to adverse microclimate for WF and improved parasite activity in the former. Sudac-K is in cv trials and may be released as a WF-resistant cv soon. A programme to develop SOL, LLHD long-staple cotton is underway.

5B—R14

RESISTANCE TO POTATO CYST-NEMATODES

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Background and objectives

Potato cyst-nematode occurs as two species Globodera rostochiensis and G. pallida causing in the U.K. an average 9% loss to the ware potato crop. Resistant cultivars, embodying genes from wild and cultivated South American potatoes, offer an alternative to chemical control. Effective resistance to British populations of Globodera rostochiensis is available in several cultivars but cultivars with effective resistance to G. pallida have proved difficult to develop. In a major source of this second type of resistance, the wild potato Solanum vernei, resistance is inherited via several genes. Some plant breeders working with this material have claimed the resistance is horizontal (or non-race specific) and therefore durable, a very valuable attribute. However some evidence existed to suggest this was not so and the objective of the work described has been to test the durability of this resistance by attempting to select nematodes able to develop on potatoes embodying genes for resistance from S. vernei.

Materials and Methods

Six nematode populations were successfully maintained on plants of one or two clones, replicated five-fold. (Some others failed to maintain themselves on the resistant clones.) Five of these six were British field populations. The cysts developing on a clone were counted and a standard quantity transferred in the following season to a new plant of the same clone and onto a fully susceptible cultivar. Comparison was made each year between numbers of cysts developing on the resistant clone and the susceptible cultivar. The cycle was repeated five times. The resistant clones, 62.33.3 and 65.346/19, are hybrids with S. vernei used as standard test lines for differentiating pathotypes of potato cyst-nematodes.

Results and conclusions

A common slope fitted to the data shows an increase of about 10% per generation on the resistant clones, compared with numbers of cysts produced on the susceptible cultivar. In field conditions the build up would not be so rapid because of carryover of unhatched eggs from one year to the next damping the selection effect. But application of a population model shows that breakdown of the resistance will eventually occur in the field. With an initial population of 50 nematode eggs/g soil, and a hypothetical resistant clone grown in a four course rotation with non-host crops in intervening years, the post-harvest population of the nematode declines at first but builds up to nearly 200 eggs/g within 8 rotations.

The results obtained demonstrate that resistance to G. pallida derived from S. vernei is not durable and can be selected against, starting with British field populations. Unfortunately preliminary evidence suggests this may be true also for the other major source of resistance to G. pallida. Fortunately selection under field conditions is likely to be a prolonged process but use of resistant cultivars, when they become available, will require careful management if their useful life is to be maximised. Furthermore the search for other sources of resistance should be maintained.

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TOLERANCE OF CEREALS TO THE CEREAL CYST NEMATODE, HETERODERA AVENAE

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Background and objectives

Tolerance of cereals to H. avenae attack generally decreases in the order oats, wheat and barley, with susceptibility increasing in the reverse order. Resistant varieties, especially of oat, often suffer the damaging effects of nematode invasion to a similar or even greater extent than susceptible varieties. Although tolerant and resistant barley varieties have been developed reductions in yield are still incurred. Breeding for tolerance to nematode attack could be an effective means of reducing yield loss and thus it was necessary to define a mechanism for tolerance which could be incorporated into a breeding programme. It has been suggested that tolerance to H. avenae is related to differences in root growth, but this aspect has not been investigated fully with reference to gross morphology of the root system. Subsequently, the effects of nematode invasion on root growth and development needed to be determined, once the tolerance mechanism had been defined.

Materials and Methods

Seeds of near-isogenic susceptible and resistant spring varieties of oat, wheat and barley (supplied by the Welsh Plant Breeding Station) were sown directly into 100g pots containing uninfested and natura-ly infested soil with an H. avenae (pathotype A) population of 35 eggs/g soil. Plants were destructively harvested at 20, 30 and 40 days after sowing. Root growth was measured by taking photocopies of the entire root systems; the number and type of laterals were counted visually and root lengths were determined using a cartographers map measurer. All other parameters e.g. shoot growth, nematode invasion were determined by established techniques. Experiments which investigated the effect of nematode invasion on root growth and the size of the host root system on invasion, used the susceptible and resistant oat and barley varieties. Seedlings were transplanted to 3cm pots and inoculated with second-stage juveniles of H. avenae (J_2). To determine the effects of plant age at inoculation, plants were inoculated with 1000 J_2 at 5, 10 and 15 days after transplanting. To assess the effect of initial inoculation density, 10 day old seedlings were inoculated with 1000, 2000 and 4000 J_2/pot. In both experiments plants were harvested destructively at two day intervals on five occasions after inoculation. All plants were grown in a Fisons controlled environment growth cabinet at 20°C day and 14°C night temperatures with an illuminance of 18,000 lux (day length 14h).

Results and conclusions

Tolerance to H. avenae invasion in oat, wheat and barley was attributed to differences in the growth rates of their root systems. It is proposed that barley roots either escape invasion by damaging levels of nematodes or tolerate invasion due to dilution of the nematode number cm^{-1} root. Barley also exhibited an inherent capability for increased root growth when invaded by H. avenae, the extent of which contributed significantly to the tolerance of the host root system. Plant age at the time of maximum juvenile invasion significantly affected tolerance of cereal roots and was also attributed to the dilution of nematode numbers cm^{-1} root in older plants. Similarly, host plants were more tolerant of the lowest inoculum level. At no time were susceptible and resistant varieties different in their responses to nematode invasion with respect to root growth.

It was concluded from the root growth analysis and data for nematode invasion that the detrimental effects of nematode attack were due to mechanical damage or impedence resulting from two independant effects (a) inhibition of root extension and (b) inhibition of lateral root production. The former occurred due to the process of nematode penetration regardless of nematode density, the latter was density dependant (number cm^{-1} root) and also dependant upon the developmental status of the individual root. Lateral root primordia originate endogenously and must traverse living cortical tissue to emerge from the parent root. At a certain nematode density cm^{-1} root, the nematodes from a temporary obstruction to advancing primordia and hence the extent of damage incurred by a host plant is entirely a result of the mechanical impedence imposed upon its root system at any given time. The damage incurred is dependant upon several inter-dependant factors, the developmental status of individual root members at the time of maximum juvenile invasion, the nematode density cm^{-1} root and the compensatory abilities of other parts of the root system.

Therefore, breeding of varieties with greater growth rates is desirable and may increase tolerance to nematode attack, particularly through increased rates of extension and early development of root systems.

5B—R16

SELECTION FOR ASULAM TOLERANCE IN CROPS

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Background and objectives

One of the requirements for a herbicide is that there should be minimal damage to the crop on which it is used. Where this is a problem such as the poor selectivity between cereals and the grass weeds, wild oat and brome, or oil seed rape and the cruciferous weeds Sinapis arvensis and Raphanus raphanistrum, there would be an advantage either to have more selective herbicides or to increase the herbicide tolerance of the crop. Since the production of new herbicides is such a long and costly process, it may be more economic to select for herbicide tolerance. In the past the selection for herbicide tolerance has involved varietal and intra-varietal screening using intact plants. However, now new techniques of tissue culture offer the possibility of screening larger numbers of individuals by using single cells or partially differentiated systems. The aim of the project was to choose a number of crop-weed situations where the selectivity was poor then attempt to increase the level of herbicide tolerance by intact plant and cell selection methods. The herbicide chosen was asulam since it was effective against broad leaved and grass weeds. It also showed the same mode of action in intact plants and in tissue cultures which meant that it could be used in a cell selection procedure. The crop species chosen were oil seed rape, barley and celery.

Materials and Methods

Intact Plants: Seedlings of 23 varieties of rape grown in seed trays in the glasshouse were sprayed with Asulox at the 2-leaf stage using a dose rate of 1.5 kg ha^{-1}, then the progeny from surviving individuals were screened again. Finally the F-1 and F-2 progeny were tested for asulam tolerance by measuring mortality and the changes in morphology in the asulam treated seedlings. Barley seedlings of a composite cross and of old and modern varieties were exposed to a maximum of 2.0 kg ha^{-1} of asulam at the 2-leaf stage under the same conditions. The F-1 progeny from surviving plants were screened again and then the asulam tolerance in the F-1 and F-2 progeny were assessed in the same way as the rape.

Tissue cultures: Callus cultures were initiated from stem explants of rape and barley. Both cultures failed to show a consistent growth pattern or the ability to regenerate plants so work was discontinued on this type of tissue culture. However embryo cultures of barley were found to be very sensitive to the presence of asulam in the nutrient medium and consequently were used to screen for asulam tolerance. Tissue cultures of celery could be subcultured routinely and the regenerative process could be controlled. The celery cultures were grown on asulam containing medium then surviving cell lines and regenerated plants were tested for asulam tolerance. Plants were grown to maturity and the seed collected for progeny testing.

Results and conclusions

Of the rape varieties tested, Norde proved to be the most tolerant. The F-1 and F-2 progeny selected from this variety also showed that the tolerance to asulam could be increased by selection. In the barley asulam tolerance was found in the composite cross and old varieties of barley but seed set was very poor, so further work was concentrated on the the modern varieties, of which Midas and Sonja were found to be the most tolerant. Progeny testing of the F-1 and F-2 by both intact plant and embryo testing again showed increased levels of tolerance as a result of selection. In celery tissue cultures selection produced asulam tolerance in cell lines and in regenerated plants. Because the celery seed derived from the regenerated plants was not viable no progeny testing could be performed. In conclusion a range of tolerance to asulam was found in varieties of rape and barley. In addition the degree of tolerance could be increased in rape, barley and celery by intact plant and cell selection methods.

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PROTOPLAST FUSION OF TRIAZINE-SENSITIVE POTATO WITH TRIAZINE-RESISTANT *Solanum nigrum*: SEGREGATION OF RESISTANCE AMONG REGENERANTS

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Introduction

One of the uses of the s-triazine resistant weeds that have appeared, is to transfer their resistance to related crops, for use in areas where triazine resistance has not, and probably will not, become a problem. This has been done by classical genetic mechanisms in rape (Souza-Machado, 1982) which are not possible for other crops. It has been suggested to transfer this resistance by protoplast fusion in related crop-weed pairs (Gressel et al 1978, 1982).

Materials and Methods

One of the maternally inherited s-triazine resistant *Solanum nigrum* biotypes that appeared in France was used (kindly supplied by J. Gasquez, INRA, Dijon). This plastome marker is being used in following plastid compatibilities in interspecific protoplast fusion experiments. Protoplasts prepared from sterile tip-cultures were fused under conditions that (potato x potato) co-fusion products did not regenerate (Binding et al, 1982). Plastid DNA was extracted from many of the clones and subclones and subjected to restriction with the DNAases XHO-1, PVU-2, BAM-H1 and BGL-1, and the DNA pieces were fractionated by electrophoresis (Gressel et al 1983).

Results

2705 clonally regenerated shoots were first screened for leaf hair characters and pigmentation and sixty clones were dissimilar from *S. nigrum*. These were tested for response to atrazine, chromosome number, and five morphological characters. Nine clones had enough mixed characters to suggest clear origin from fusants. Segregation between resistance and sensitivity occurred during regeneration of 13 clones, including 6 having a reversible chlorotic leaf mosaic in the presence of atrazine. Pieces of these segregants were subcloned to stably sensitive and resistant plants (Binding et al., 1982).

As triazine resistance is inherited on the plastid DNA, we assayed for the plastid parentage of triazine resistant and sensitive regenerants using restriction endonucleases. This was needed to ascertain whether mosaics and similar phenomena were due to a back mutation to sensitivity or due to late segregation of the plastids in the mixed fusion products.

Plants of susceptible (wild type) and resistant biotypes of *S.nigrum* had the same restriction patterns with all four enzymes. Potato had different patterns from *S. nigrum* with all except PVU-2. All plants regenerated from protoplast fusions that were triazine resistant had the *S. nigrum* patterns, whether they were more morphologically similar to *S. nigrum* or potato. All regenerant clones that were triazine sensitive had the potato DNA restriction patterns whether originating from a primary clone or a segregated mosaic. This potato pattern was even found in a triazine sensitive clone with a strong resemblance to *S.nigrum*. This clone was clearly not a revertant to sensitivity, but a segregated fusion product (Gressel et al 1983). Methods are now being used for fusion that will preclude expression of the *S. nigrum* nucleus in the fusion product.

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5B—R18

DISTRIBUTION ANALYSIS OF THE PROPANIL-RESISTANCE GENE IN THE GENUS ORYZA

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Background, objectives and methods

Usual cultivated rice plant (*Oryza sativa L.*) has a special enzyme, aryl acylamidase I, which can hydrolyze a rice herbicide, propanil (3', 4' - dichloropropionanilide) into 3,4-dichloroaniline, and is very tolerant to the herbicide. In this study, in order to clarify the susceptibility of weedy rice plants to propanil and also to get new information on genetical relationships among the Genus *Oryza*, the distribution of this enzyme in the genus, including the cultivated and wild (weedy) rice plants, was surveyed by measurement of the enzyme activity of leaf homogenates from each rice plant. Also the resistance or susceptibility of these rice plants to propanil were tested by foliar application of the herbicide in a practical dosage.

Results and conclusions

Distribution of the enzyme in the Genus *Oryza*, *Rhynchoryza subulata* and barnyardgrass (*Echinochloa oryzicola* Vasing.) is shown in the following table.

TABLE
Distribution of aryl acylamidase I (propanil hydrolyzing enzyme) and susceptibility to propanil

Section	Species	Genomes	Enzyme*	R or S**
Oryzae (Sativae)	*Oryza sativa*	AA	+++	R
	(susceptible mutant)	AA	-	S
	O.sativa spontanea	AA	+++	R
	O.perennis	AA	+++	R
	O.glaberrima	A^gA^g	+++	R
	O.breviligulata	A^gA^g	+++	R
	O.australiensis	EE	-	S
	O.eichingeri	CC	+++	
	O.punctata	BB	++	R
		BBCC	++	R
	O.officinalis	CC	++	R
	O.minuta	BBCC	+++	
	O.malabarensis	BBCC	++	R
	O.latifolia	CCDD	+	R
	O.alta	CCDD	+	R
	O.grandiglumis	CCDD	+	
Ridleyanae	*O.ridleyi*	????	+?	
	O.branchyantha	FF	-	S
	O.perrieri	??	-	S
	O.tisseranti	??	-	
	Rhynchoryza subulata (*Oryza subulata*)	??	++	R
	Echinochloa oryzicola		-	S

*The activity was assayed with the 4th and 5th leaves at the 5th leaf-age; +++: higher than 80%, ++ 50-80 %, + : less than 50% of the activity of *Oryza sativa*, -: no activity.
** R:resistant, S:susceptible, 7-10 days after application of 0.35% (a.i.) propanil emulsion

It was concluded that the rice species having genomes relating to A,B,C and D have propanil hydrolyzing enzyme and are tolerant to the herbicide. On the other hand, rice species having genomes E or F lacked the enzyme activity and those without the enzyme are susceptible to propanil. In general the weedy rice species of the Section *Ridleyanae*, e.g. *Oryza perrieri* or *tisseranti* in which genome analyses have never been done, have no such enzyme and are susceptible to propanil.

Rice species having genome D showed relatively lower activity of the enzyme. Usually these species show lower content of chlorophyll in the leaves. The experiments to solve whether the lower activity of the enzyme in these species comes from the lower content of chlorophyll or from the characteristics of genome D is in progress.

These genetical characteristics may give fundamental information for breeding rice tolerant to pesticides.

An exceptional information that *Rhynchoryza subulata*, syn. *Oryza subulata* contains the enzyme and is tolerant to propanil will offer a problem to the classification of the Genus *Oryza*. The propanil hydrolyzing enzyme in *Rhynchoryza subulata* will be compared with that from *Oryza sativa* as to the enzymatic properties.

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REACTION OF WHEATS TO EAR-COCKLE NEMATODE

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Background and objectives

Wheat diseases surveys reported widespread occurrence of ear-cockle nematode in the states of Himachal Pradesh, Punjab, Haryana, Rajasthan, Delhi, Uttar Pradesh, Bihar and Madhya Pradesh. Control of this nematode through floatation technique is well known, but continued occurence and spread of this nematode demonstrates the non-use of this method. Use of resistant cultivars is perhaps the only answer. Reaction of some of the available cultivars and Triticum spp. was therefore studied.

Materials and Methods

Seventy three wheat cultivars and seven Triticum spp were tested for their reaction to ear-cockle nematode both in field as well as in pots. Inoculations were done at the time of sowing by placing three galls of the nematode with each seed. Each variety was replicated thrice. The disease incidence was recorded at the harvest time when individual ears were examined for infection.

Results and Conclusions

Under field conditions 12 cultivars showed resistance. Testing in pots showed that 6 of these were escaped. The rest, six cultivars namely Sonora 63, Sharbati sonora, E 5008, E 5075, E 6160, and HD 1633 showed resistance and these could be used in breeding programmes.

Both under field and pot tests, Triticum durum, T. Dicoccum and T. Compactum showed susceptibility to the nematode. None of the rest, four species namely T. Turgidum, T. Vavilovi, T. macha and T. spelta produced ear-heads in pot and field tests. The reaction of these species, therefore, could not be ascertained. T. durum and T. Compactum are new records of susceptibility to this nematode.

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5B—R20

PROTECTION AND BREEDING STRATEGY AGAINST VIRUSES OF SUGAR BEET

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Background

In some years one or more viruses are present in more than 80% of sugar beet plants in Czechoslovakia. Efforts to breed resistant or tolerant cultivars are considerable. The conventional growing of beet mother plants is the largest source of infection. Direct sowing as an alternative is a major endeavour of our integrated protection programme.

Materials and Methods

Pollen from healthy and virus-infected plants, was collected during cloudy moist weather and sown onto a saccharose/peptone agar with pH adjusted to 5.4 with citric acid. Disks of 1,5 cm diameter were cut out and placed in Petri dishes containing wetted filter paper at 25± 1 °C. Germination was assessed within 2-3 hours. Beet mother plants from direct sowings and from conventional transplanting were observed at many State Farms. Their over-wintering ability was observed and their health in the first half of June, classified as healthy; mosaic; yellowing virus or mixed virus infected.

Results

The germinating capacity of sugar-beet pollen was slightly reduced by beet mosaic (1.4%). Beet yellowing viruses caused a greater reduction (15%) and mixed virus infections a reduction of 25%. The health and seed yields of sugar-beet mother plants planted conventionally or by direct sowings bwetween 1979 and 1982 were evaluated. Between 29-49% of direct sowings were killed during the winter due to late sowing (September) or poor husbandry. Surviving plants were healthier (1.1 - 1.3% infection compared with 35-60%) and gave higher seed yields.

Discussion

Pollen viability of monogerm varieties is higher in direct sown seed crops because the incidence of viruses is less. The technique is simpler and fungal diseases reduced also. Sugar-beet is a relatively new crop. To increase root weight and sugar content, it is sown in the early spring. But this exposes the young plants to the period of the greatest immigration of aphids, Myzus persicae and Aphis fabae and to mosaic and yellowing viruses. The wild, parental forms are not exposed to such an infection pressure as the cultivated forms.

Breeders are endeavouring to produce cultivars resistant or tolerant to viruses. Some valuable progress has been made but, usually, to the detriment of sugar content and purity. At present, there is little hope of introducing more tolerant or more resistant cultivars that would solve the problem of virus diseases. Traditional storing of watery beet roots, required for seed production, in beet clamps over winter excluded natural selection for frost resistance and could carry over a strong source of infection. We regard Over-wintering of healthy beet crops in the field is highly important, both theoretically and practically. The occurrence of virus diseases in beet mother plants also greatly influences the healthiness of the sugar-beet root crop. The health of the root crop is substantially better in Central-and East-Slovakia, where beet mother plants are not produced, than in the region of West-Slovakia where seed is being grown. If seed growing in the conventional way were excluded, and replaced by direct sowings, there would be a great improvement in the health of the root crop.

The reduction of the source of infection is one of the basic principles of beet protection against viruses. Introducing direct sowing of the beet mother plants must also be supported by selection of types that tolerate over-wintering in field conditions, an aspect of breeding that has to far been completely neglected. Agronomical research, especially sowing date, and research on frost resistance must play their role in introducing direct sowings.

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