SESSION 1

THE FOURTEENTH BAWDEN LECTURE

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CROP IMPROVEMENT: CONSTRAINTS AND CHALLENGES

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I met Fred Bawden on one or two occasions early in my career in the Agricultural Research Service. He was Director of Rothamsted at the time, a rather awe-inspiring virologist to the callow, inexperienced plant pathologist/geneticist that I was at the time. There are many stories about him. The only one I can add was his flouting of the political conventions of the time by plastering election posters all over his car and parking it prominently in the Rothamsted car park, to make it clear to everyone which Parliamentary candidate he was supporting. At conferences and scientific society meetings he relished controversy and deflating pompous egos. He was a major figure in the development of British agricultural research, and it is a great honour to be asked to contribute a lecture in the series bearing his name.

The agrochemical industry clearly has crop improvement as one of its major objectives. Until fairly recently, the industry has pursued this through the products of its chemical and biochemical research. There is of course another way of improving crops, namely through plant breeding, and as you might expect, this is the subject of my lecture. Biology, and more particularly genetics, is on the crest of a new and rising wave which we can loosely call biotechnology. I am tempted to try to shock you by saying I am glad I am not a chemist trying to invent new products in the laboratory, because I will be out of a job in a few years as genetic engineers begin to build an innate pest resistance, making the kind of applied chemistry that the majority of you are concerned with irrelevant. But the cynics among you will reply, "We have heard it all before and we are not going to sit up and listen until the biotechnologists have products growing in farmers' fields".

The correct attitude to adopt about the impact of biotechnology on crop improvement is probably between these two extremes. The sustained and increasing interest in plant breeding among agrochemical companies is a clear indication, at least to me, that they recognize the threat and I see among even the more cynical plant breeders a growing interest in the speed, convenience and accuracy of new tests and methods, resulting from current research. In the 1984 Bawden lecture, Dr. Mary-Dell Chilton described the principal methods for introducing foreign DNA into plants, and discussed the opportunities and prospects for producing

new crops, and the problems and difficulties that we might expect to encounter through the new technology. I will first spend a short time reviewing some examples of progress since 1984, and then go on to discuss the likely impact of two other factors namely regulation and the changes that are affecting the way plant breeding is structured. My remarks are based on my work over the last 8 years at the Plant Breeding Institute in Cambridge. I gratefully acknowledge my indebtedness to my former colleagues at PBI who helped to educate me in the complexities of the agricultural technology transfer.

Crop Improvement Objectives

Just as pesticide and herbicide chemists work to identify new compounds that are cheap, safe and effective, so plant breeders work to deliver to farmers new varieties with increased yield, improved quality and lower production costs. The breeders' goal for the major agronomic crops in the U.K. is to produce varieties that will appear on the NIAB Recommended Lists. However, rising costs and problems associated with new breeding technologies are complicating the development of new varieties.

In Britain about 80% of the wheat and barley drilled each year is bought in fresh by farmers. We are told that steady sales of seed cleaning machinery, and the ready availability of contract seed cleaning services will encourage the use of home-saved seed. Since the commercial cereal breeders depend on the royalties collected from seed sales there is an understandable interest in hybrid seed. This must be bought anew each season if the farmer is to continue to benefit from increased yield. Chemical male gametocides, or hybridising agents (CHAs), have brought the prospect of F1 hybrid seed of wheat and barley much nearer. But there are still difficulties. The first is to ensure reliable large-scale production of hybrid seed of a satisfactory level of hybridity (at least 90%) in Britain. Not only does the timing of the gametocide application have to be accurate but it must also be carried out under appropriate weather conditions. For example, rain within 6-8 hours after the application renders it ineffective.

Members of BCPC are only too familiar with the high cost of registering agrochemicals. One very promising gametocide has already failed registration in the USA. Others have not yet been submitted because of the financial risk.

Seed costs present another problem. How much will the farmer be willing to buy? It is easy to calculate that if the price of F1 hybrid seed of winter wheat to the farmer is double that of ordinary seed, the farmer will expect an increased yield of about 6% to cover his increased seed costs. Breeders are fairly confident of providing increased yields of at least 10% through the best hybrids. Other problems include the need to breed for such characters as abundant pollen production by the male parent, and reliable response to the gametocide with no effect on

female fertility in the treated parent. Many of these difficulties, especially that of seed production, may be solved by selling F2 rather than F1 seed. Providing parents with similar height and quality characteristics are used, the F2 population should be sufficiently uniform. Although it would only delivery approximately half the increase in yield of the F1 this could still be advantageous.

New Technologies to Aid the Breeder

Like any designer of new consumer products, the plant breeder has to maintain all of the positive attributes that characterize the old varieties while effecting improvements in the new ones. His task is like that of a juggler who strives to keep more and more balls in the air. Restraints on available field and greenhouse space limit the sizes of the plant populations that can be grown. There are rarely sufficient resources to employ enough skilled staff to do the increased work in the time available.

Breeders are very interested in technology which can expand their capabilities. I will cite two examples from wheat improvement at the Plant Breeding Institute. The first is the use of polyacrylamide gel electrophoresis to identify the glutenin subunits present in wheat endosperm which contribute to baking quality. The ability to recognize these protein subunits, coupled with knowledge of their genetic control, enables breeders to select parents for crosses that have complementary qualities, and to screen progeny for plants which recombine the best of both parents. The Plant Breeding Institute received its fourth Queen's Award for Technological Achievement in 1987 in recognition of the work by John Bingham and his team in improving breadmaking quality in British wheats. Peter Payne also received the 1987 Royal Agricultural Society of England Research Medal for his work on the identification of protein subunits that contribute to baking quality.

The second example is the introduction of the winter wheat Rendezvous, with a new high level of resistance to eyespot disease derived from the goat grass Aegilops ventricosa. The original work to transfer the alien gene into cultivated wheat was done in France, but the subsequent breeding work was carried out at PBI. The gene in question is carried on chromosome 7D, and is recessive and therefore not easy to follow in the breeding programme. However, its presence can be detected in segregating families by testing for an enzyme isomer in plant extracts. The gene controlling this enzyme activity is very tightly linked to the eyespot resistance gene on the same chromosome arm. The pedigree of Rendezvous indicated that in each cross, starting with A.ventricosa, the resistant parent had been used as a female. A comparison of restriction digests of DNA prepared from chloroplasts of Rendezvous, A.ventricosa and eyespot susceptible cultivated wheat showed that Rendezvous chloroplast DNA is the same as that of A.ventricosa, but differs from other wheat varieties. Evidently the chloroplasts of

Rendezvous come from A.ventricosa and not from cultivated wheat. It will be important to establish whether the cytoplasm has a role in eyespot resistance in this variety and, if not, to see if yield and agronomic performance of eyespot resistant lines could be improved by substituting normal wheat chloroplasts for those from the goat grass. This facet of wheat breeding could only have been explored by using the tools of plant molecular biology.

An increasing number of such tools are under development. These include DNA probes for detecting the presence of viruses and other pathogens, and to detect innate differences between lines and varieties that can be used as genetic markers. Although these may eventually be useful in distinguishing between varieties for plant royalty purposes, they provide a much more powerful means of genetic analysis that will be useful to the breeder. I will briefly review how the method is used.

DNA is extracted from a small tissue sample and digested with a restriction enzyme. The enzyme recognizes a particular sequence of nucleotide bases and cuts the large DNA molecules everywhere that the sequence occurs. Although it produces many fragments that vary in size, the cuts are very precise and repeatable. When the fragments are separated by electrophoresis on a gel and stained so that they can be seen, they form a smear. However, if the smear of fragments is blotted onto a nylon membrane, heated so that the DNA becomes single stranded, and allowed to hybridize with a radiolabelled nuclear DNA probe that is also single stranded, those fragments that carry an exactly complementary sequence hybridize with the probe. The hybridized probe DNA becomes bound to the membrane so that the unhybridized probe may be washed away. When an autoradiograph of the membrane is developed it reveals a few labelled bands representing DNA fragments that contain sequences hybridized to the probe. Digests from different individuals compared using the same probe frequently show differences in the positions of one or more of the labelled bands. These reflect genetic differences in their DNA. For example, if a base change occurs at a restriction site the enzyme will not cut at that point and a larger fragment will result. This will be revealed as a slower band on the gel. A small deletion of DNA between two restriction sites would reduce the size of the fragment and so create a faster band. Each pattern is distinct and reflects a genetic difference between the individuals which is detectable by that probe and restriction enzyme. This difference is a marker which is as useful to the modern geneticist as the pea mutants round vs. wrinkled, yellow vs. green, or tall vs. dwarf were to Gregor Mendel.

The variation in fragment size is called restriction fragment length polymorphism (RFLP). It is fast becoming a powerful tool for creating linkage maps and recognising individual chromosome segments.

The method is rapidly generating information that until now has taken lifetimes of work to accumulate. The most useful probes are those representing sequences present in low copy number in the genome. Since the DNA sequences for important genes show a large degree of conservation among different organisms some probes are useful across a range of plants.

A dramatic example of the use of this technology is in forensic medicine and human genetics, where an individual's identity and kinship can be concusively proved from the DNA in a blood sample. The method has already been applied to birds, plant pathogens and other organisms and will be invaluable for studying population genetics.

Releasing the Products of Biotechnology

Although we often complain about regulations and the constraints they impose on personal freedom, they nevertheless protect both the public at large, and the product or activity that is being regulated. Sensible and effective regulation removes uncertainty, clarifies where responsibility lies and allows planning and investment to proceed with confidence. The process of introducing regulation is often painful, slow and controversial. Recombinant DNA biotechnology is a case in point.

In 1976 scientists drew attention to the prospect that recombinant DNA techniques would allow unprecedented opportunities for directed genetic change and posed the question of whether they were a threat to the safety of the experimenters and the communities where they worked. With the help of voluntary guidelines work continued in the UK, the US and a number of other countries. Safety tests, with animals and human volunteers, showed that genetic stocks of the bacterium *E.coli* manipulated by these means posed no new hazard to human health. The guidelines required that work with pathogens, including those of plants, should only be carried out under stringent containment conditions and on a small scale. They also stated that no plant, animal or microbe containing recombinant DNA could be released in the environment.

Although the guidelines established by the Genetic Manipulation Advisory Group (GMAG) in the UK and by the National Institutes of Health (NIH) in the US encountered criticisms, they were flexible, they could be amended and above all they provided a framework for experiments to continue. They also reassured many scientific lay people who were concerned about the possible risks.

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During the last three years the pressure for environmental release has increased greatly to enable tests of newly engineered forms to be carried on outside the laboratory. In the US the tests include ice-minus bacteria for frost-resistance, glyphosate and atrazine resistant crop plants, and rhizosphere bacteria and plants containing the delta endotoxin gene fromm Bacillus thuringiensis. The product of this gene is a protein that is toxic to lepidopteran larvae. Another form of the toxin is lethal or inhibitory to coleoptera. Preparations of the bacterium have been used as the insecticide Bt over many years and on a very large scale, thus providing added assurance of the lack of any potential environmental hazard. In the UK tests include limited release of a baculovirus that attacks the pine beauty moth carrying a short non-coding DNA sequence as a marker and, at PBI in 1987, a field tests of some 100 independently transformed potato clones. These clones carry a bacterial gene for kanamycin resistance together with a second bacterial gene for the enzyme glucuronidase (GUS) coupled to a potato controlling element, or promoter, which limits expression of the GUS gene to tuber tissue. GUS activity is readily detected even in single cells when the suppressed fluorescence of certain substrate molecules is released by the enzyme cleavage of an attached glucose residue.

Permission for tests in the US and UK has required assurance that there is no likelihood of the introduced genetic information spreading through seeds or plant parts or, via pollen, to other plants of the same species or to related wild species. For the PBI experiment it was necessary to remove flower buds and, as a further precaution against spreading tubers, to carry on all cultivations using hand tools rather than machinery.

It is still too early to comment fully on the results of these field tests. In the US useful levels of herbicide and insecticide resistance have been reported but there is little or no information on the impact of transformation on yield, quality and other characters of agronomic importance. The PBI experiment showed the importance of controlling and eliminating undesirable somaclonal variation. Reliable indications of the agronomic effects of the selective marker employed in tissue culture will not be available until field grown tubers can be evaluated in subsequent trials so that tubers of treated and control clones are of approximately similar physiological age.

It seems clear that the immediate products of transformation are unlikely to be useful as finished varieties. They are more likely at first to be a new form of germplasm for the breeder to use in conventional breeding. This is because there is at present no means of controlling either the number of copies of new genes nor where in the recipient genome they will be integrated.

Regulating Product Release

British plant breeders have had little experience of regulation other than the need to conform to the standards of distinctiveness, uniformity and stability (DVS) that are part of the plant variety rights scheme administered by MAFF in the UK. The first two standards protect the breeder from others infringing on his royalty rights to named varieties on the National List. All three standards offer assurance to the farmer that the seed certification label accurately described what is in the bag of seeds he buys.

To make the scheme work to the satisfaction of the testing authorities breeders have to spend time and effort on final polishing particularly with regard to uniformity. There is no question that this has delayed, some would say unnecessarily, the availability of improved varieties to farmers. This is because new entries for National List tests have to be resubmitted if they do not meet the standards and because scarce resources must be devoted to obtaining levels of uniformity that add no agronomic or end user value to the variety.

The National List trials that establish value for cultivation and use are of more value to the farmer since they compare, over two years, new varieties with each other and with well-known standard varieties. The more promising of the new entries are selected for a third year of trials by the National Institute of Agricultural Botany to establish an elite Recommended List. Although only a voluntary scheme run for the benefit of NIAB fellows it has set such high standards for the industry that to achieve significant commercial success a variety must be on the NIAB Recommended List. Removal from the list indicates that the variety has either become outclassed or has a defect that was not detected in the initial trials.

In the USA during the 1950's a late blight resistant potato variety named Linape had to be withdrawn after being released because its tubers had unusually high levels of alkaloids which made certain people who ate the cooked tubers ill. All new potato varieties are now tested for alkaloid content to ensure the tubers meet quality standards that protect the consumer. Another example of current interest is the new standard for erucic acid and gluosinolates soon to be implemented by the EEC in so-called "double low" varieties of oilseed rape.

The mechanisms for regulating environmental release in Britain and the US are currently in a state of flux. Authorities are proceeding cautiously, allowing limited release in order to generate experience and establish procedures and criteria based on precedent. Considerable relaxation of the originally very stringent laboratory guidelines occurred as a result of case-by-case deliberation. Most scientists support a similar approach with respect to environmental release. A major source of difficulty is the much larger number of interested parties and regulatory authorities that can become involved.

The recent example during the summer of 1987 of an unauthorized inoculation of elm trees with what at first appeared to be a laboratory produced recombinant strain of *Pseudomonas syringae* to control Dutch elm disease in Boseman, Montana was unfortunate. The incident clearly showed once again the extent to which the media are prepared to document

any apparent infringement of the regulatory process and to crucify the individuals concerned adding unnecessarily to the public's anxiety.

The establishment of case law and the successful handling of examples where perhaps unduly elaborate precautions are taken will be necessary to build public confidence. As the regulatory authorities and committees gain experience and information it should be possible to have an increasingly accurate assessment of risk. In this way the procedures for environmental release could be modified so that they are no more burdensome than for releasing the products of conventional breeding programmes.

Of particular interest will be the development of constructs that restrict the expression of introduced genes to tissues that are not consumed. For example, Bt toxin production might be limited to roots, stems and leaves so that fruits would not contain it.

The Changing Role of State Breeding in Britain

The introduction of plant variety rights in 1961 and the development of a vigorous private plant breeding industry in Britain soon raised the question of how state supported and private breeders could coexist. Was it fair to use taxes to support a state organization that competed for the same market? The private breeders argued for the system used in Holland whereby state breeders would produce only advanced lines for distribution to the industry and would be prevented from releasing finished varieties. Her Majesty's breeders, as Sir Joseph Nickerson was wont to call us, argued that release to farmer's provided the ultimate test-bed of their research and development ideas. The direct contacts with farmers, the seed trade, millers, maltsters and processors gave direction and impetus to the work that in their view was missing in the Dutch scheme. Also the kudos and recognition attached to success was an important stimulant to further effort.

In the early eighties, the ARC made an important concession to these pressures by agreeing that for vegetables its breeders would only release new varieties that demonstrated new principles such as new plant types or new forms of pest and disease resistance. Less dramatic improvements would henceforth be released as advanced lines to private companies. Much more severe pressure was to come in 1984 and 1985 as ARC now renamed AFRC (Agricultural and Food Research Council) had to adjust to major cuts in funding. Staff redundances were introduced that resulted in some 600 staff being forced to leave the research service. At PBI a number of programmes had already been stopped including breeding forage maize, red clover, lucerne and durum wheat. These moves culminated in the closure of the Sugar Beet Department in March 1986. Other work on aphid resistance in cereals and research on the development of the cereal apex was also stopped and engineering and electronic workshops were closed. The triticale breeding programme was identified for closure but a private sponsor (Semundo, Ltd) agreed to take over the funding of this work in 1985 on a renewable five-year contract, thus acquiring a major share in the rights to any triticale varieties produced after the contract date.

As a result of these and other measures, PBI faced 1986 confident that its major programmes could continue unimpaired. However, AFRC had also to accommodate to a further massive cut in MAFF support for R & D totalling £20m which was to be born by the Agricultural Development and Advisory Service (ADAS) and AFRC during '85-'86 and '86-'87. A research priorities board was established to provide advice on what to keep and wht to jettison. This board recommended seeking industry support for certain R & D activities including plant breeding.

In the meantime, MAFF had begun to analyse the prospect for finding a purchaser for the National Seed Development Organization (NSDO), which they had set up soon after plant variety rights were established in the sixties. They soon realized that unless NSDO was assured of a continuing flow of improved varieties it could only become a wasting asset of comparatively little value as its portfolio of varieties became out of date. The stage was thus set to engage a merchant bank, Lazard Brothers to establish the commercial viability of selling the principle source of NSDO's new varieties, the PBI, together with NSDO as a going concern.

During the lean years of redundancies and cuts the breeders had seen the basic science programmes expand through the influx of external funding to support work in molecular genetics, biotechnology and cytogenetics. Although some investment was made in plant breeding by redistributing scarce resources the breeders saw privatization as providing the most likely opportunity for significant additions that would help them to retain their comparative advange. Even so they were worried by the prospect of losing direct access to the research and science support "down the corridor".

Unfortunately, the results of research in basic science do not apply themselves automatically to solving other people's practical problems. The benefits from biotechnology will come most rapidly by building bridges between breeders and molecular biologists. Direct exchange between these two kinds of scientists is possible but difficult. They use different technical vocabularies, work to entirely different time scales, and don't read the same journals or attend the same meetings. This technology transfer calls for the establishment of a special cadre of scientists who can work with both groups. They must understand the breeders goals and the constraints to rapid progress in attaining them. At the same time, they must be conversant with current research in molecular biology and collaborate in the production of materials that are of direct interest to breeders. The field test of kanamycin resistant potato clones at PBI earlier this year is a case in point. Its establishment rested heavily on the work of a small team that used the Agrobacterium Ti vector to transform potato leaf discs, regenerate plants and bring them to the poiont where they could be planted in a field. An equally important part of this work was the preparation of the application to the Advisory Committee on Genetic Manipulation for agreement that the field test could go ahead. In this way an experiment of considerable interest to both groups was set up.

Other changes were taking place. In 1983, AFRC entered into a research and marketing agreement binding six of its institutes, including PBI, to pursue the exploitation of certain products of their research in biotechnology through the Agricultural Genetics Company

(AGC). The contract imposed constraints, confidentiality and procedures to protect patent rights that proved to be difficult for staff to accept and which at times proved disruptive to the establishment of research contracts with other companies in AGC's field of interest.

Scientists in industry are used to these constraints. Imposed restrictions were irritating to people used to sharing information and materials for the better and faster advancement of science as they saw fit. In fairness to AGC this is a trend that many see as inevitable in both Universities and publicly funded research institutes. It arises from the need to protect commercially valuable information. It presents the challenge of finding mutually acceptable ways to continue collaboration and exchange because without this interaction only very large laboratories can be self-sustaining for long. To be cut off from the flow of ideas and materials by failing to give as well as take is to commit scientific suicide. Unfortunately this same trend is beginning to influence and temper the generosity of the wealthier nations towards the developing world and may interfere with and slow down aid to developing agriculture.

The sale of PBI and NSDO was a political decision taken because of the government's conviction that a state organization that was profitable would be still more so under private ownership. Political sympathy for investment in agricultural research was and still is quite severely dampened by exasperation with the surpluis of produce in the European economic community. As one of my former colleagues recently put it, "the enormous mounds of surplus wheat are a monument to the efficiency of plant breeding". The cause of overproduction is not plant breeding but rather the economic system and support prices that encourage and sustain it. Although politically embarrassing, the present agricultural surplus will surely prove to be of short duration in terms of scientific lifetimes. It would be a tragic error to further dismantle and weaken the state supported base for agricultural research in the UK by regarding it as a luxury we neither need nor can afford. It will continue to be of vital importance in sustaining future national production of food and fibre. Nations must have these materials and consequently will always require research to improve production efficiency and quality and properly control the impact of agriculture on the environment.

One of the most important consequences of the decision to privatize PBI NSDO could be the disruption of the partnership between breeders, molecular biologists and cytogeneticists. Although I have always maintained that the decision to privatize PBI was mistaken, I am confident that the new structures to replace it that are now being planned can eventually provide a comparable service to crop improvement. The PBI staff who will remain within AFRC and who are expected to move to Norwich in 1990 as part of the AFRC Institute for Plant Science Research will have to forge new connmections with private plant breeders. The complications of patent and know-how protection must not be allowed to restrict their dialogue. The PBI breeders will have the opportunity to enlarge their horizons by applying their skills to a wider marketplace than the UK. They are not afraid of being judged and rewarded according to their success in this arena. They will have hard choices to make over where to invest resources and how to maintain their access to the AFRC science developed to service their needs.