BIOTECHNOLOGY AND CROP PROTECTION

A WILSON*, AECI Limited

The agricultural sector of the South African economy contributes a large proportion of the gross domestic product with the main crops being valued at several thousand million rands per annum. During recent decades tremendous improvements in crop yields have been achieved in South Africa despite the adverse problems of losses due to diseases, pests and drought. However significant crop losses in excess of R1 000 million per annum are often recorded and faced with the task of increasing production and efficiency the farmer must limit these losses.

Traditionally, the farmer has adopted a number of approaches including good farming management practices, crop breeding programmes and the use of fertilisers, herbicides and pesticides. For example, in 1987 the annual agricultural chemical market for herbicides, insecticides and pesticides was in excess of R350 million. Despite this major investment by the farmer, annual losses amounted to several hundred million rands. Biotechnology, and particularly molecular biology, can help the farmer restrict these losses. Economists studying the development of biotechnology concur with this prediction. They predict a change in the farmer's budget from fertilisers and pesticides towards seeds. In 1986 in Europe the wheat producers' costs included:

Seeds	20%
Fertiliser	45%
Sprays	35%

In 20 years' time the seeds are predicted to account for more than 50% of his costs.

The chemical companies agree. During the past 10 years chemical companies have spent more than R25 000 million in buying up seed companies. Meanwhile these companies are investing large monies in research and development to establish biotechnology as the vehicle to produce not only seeds and plants but a whole new array of biological products for enhanced crop protection and production.

Application of Biotechnology

Biotechnology encompasses a whole array of interrelated technologies. Many of these technologies have been harnessed in tackling the scientific hurdles involved in production of plants and seeds with resistance to pests, diseases and herbicides.

Any new biocontrol research venture must focus on an area where the current state of the technology and the needs in the market place converge, allowing realistic objectives to be set. This applies not only to the selection of crops for which the genetic engineering technology is already developed but also to the selection of the disease or pest to be tackled, since the route towards developing crop protection products depends to a large extent on the particular disease or pest involved. The diseases, pests and biocontrol routes available can be summarized as follows:

Biological Control of Pests

Biological pesticides have now been recognised as a factor in crop protection and insect vector control. These pesticides are natural, disease causing microorganisms such as viruses, bacteria and fungi which are capable of infecting specific pest groups. Their narrow range of activity makes them highly specific to certain pests and safe in the environment.

Bacillus thuringiensis is the most successful of these toxins so far used, having been found effective against for example caterpillars, corn borer and gypsy moth. It generally acts through poisoning the stomach with the combined actions of a protein toxin and septicaemia due to germination of spores and bacterial growth within the insect. Uses of Bt as an insecticide range from forestry to vegetables, corn and fruit trees. Sales now exceed R75 million per annum.

These insecticidal toxins are proteins known as endotoxins. Interestingly the genes for endotoxin production have now been cloned. This now gives the molecular biologist the opportunity to improve the potency, the host organism and the range of activity. For example, in 1984 Monsanto scientists succeeded in getting the relevant genes into a corn root colonising bacterium. As a result the insecticidal crystals are found beneath the surface of the soil where the feeding insects do the most damage.

Another new exciting development has been to introduce this gene into the genetic make up of the plant. As a result plant tissues themselves produce the relevant toxins and the leaves are toxic to the relevant insects.

1. *Fungal Diseases*
   Fungal diseases such as potato blights, stalk and root rots are probably best combatted through identification and application of other non-pathogenic fungi or bacteria which compete with the pathogen and may produce toxins or other antifungal compounds. However this is somewhat of a hit-or-miss affair and formulation of a commercial product to provide long term plant protection could present problems.

2. *Bacterial Diseases*
   Like fungal diseases, biocontrol of plant pathogenic bacteria (which cause diseases such as bacterial

**Assistant Research Manager (Biotechnology). Research & Development Department*

wilt in tobacco) depends largely on identification of antibiotic substances produced by other micro-organisms. Such antibiotics are usually not proteinaceous in nature and therefore do not lend themselves to a genetic approach. Again there may be problems in finding some method of application of a commercial product.

3. *Biological Routes to Herbicide Resistance*
Much interest is being expressed overseas in the development of herbicide resistant plants. Many herbicides are of limited use because they kill the cultivated crop as well as the weeds. This is true of some major herbicides such as 'Roundup' (active ingredient glyphosphate, or N-(phosphonomethyl) glycine). There is therefore considerable demand for crop plants with glyphosphate resistance. Scientists at Monsanto (the manufacturers of 'Roundup') have engineered glyphosphate resistance into soybeans, cotton, sugar beet and petunias. The target enzyme for glyphosphate is 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase. A mutant form of the enzyme which is insensitive to glyphosphate has been engineered into the plants.

An alternative approach to generating herbicide resistance would be to identify enzymes capable of degrading the herbicide from organisms in the soil. The genes for such enzymes would then be cloned into the plants.

4. *Viral Diseases*
There are a number of possible routes to biocontrol of viral disease, all of which are approached through gene cloning techniques:

4.1. **Agrobacterium route**
Dicotolydenous plants are susceptible to infection with the tumour inducing bacterium *Agrobacterium tumefaciens.* When this pathogen infects a plant it inserts a fragment of its own DNA into the genetic make-up of the plant cell. Using this same segment of DNA and firstly inactivating the tumour inducing instruction and then secondly attaching an appropriate piece of DNA to it we now have a vehicle for inserting relevant genetic material into a plant. This process is conducted in a test tube and has been successfully applied to a whole series of crops such as potatoes, tobacco and tomatoes. Unfortunately this process has met with little success in the monocot plants such as maize.

4.2. **Direct uptake**
Another, perhaps less elegant route involves removing the cellular walls with enzymes and incubating in a test tube with foreign genetic materials. Uptake is encouraged by electric shock.

4.3 **Injection techniques**
Appropriate cells are immobilised as protoplasts and then injected before reculture and regeneration.

5. *Regeneration of Whole Plants and Pests*

Many crop diseases and pests are now being tackled and useful transgenic plants and seeds produced. However the economically important cereal crops such as maize are another matter. These crops have been found to be particularly recalcitrant to regeneration from single cells essential to the techniques already described for cellular and molecular manipulations.

Even after useful transgenic plants have been obtained they must undergo extensive conventional breeding and selection trials and also field tests under regulatory conditions.

In order to regenerate whole plants, the 'transformed' cells must be induced to regenerate into whole plants, ensuring that the engineered trait is inherited. There are two approaches. Firstly by organogenesis, where the cultured tissue produces a shoot which is excised from callus tissue and placed in a rooting medium before transplanting to the soil. The other method, somatic embryogenesis, involves the formation of somatic embryos from non-reproductive cells by incubating in special media designed to induce growth of complete plants. Later seed is collected and checked for the desired trait.

Stability of the new trait must then be tested for several generations before limited field trials, patent establishment and legal clarification.

Potential Crop Protection Products
The new genetic techniques in biotechnology hold much promise; their substance will be proved as new products become available to the farmer. Some interesting examples of potential new products are detailed below:

1. *Herbicide Resistance in Plants*
Any company which is able to offer to the farmer both a herbicide and herbicide-resistant crop seeds is likely to be profitable. A wide range of crops resistant to

- triazines
- glyphosphate
- paraquat, etc.

is already under development by more than 100 companies. In fact these are likely to be the first large-scale commercial products. Many of the problems of herbicide application and soil carry-over could be problems of the past.

2. *Improvement of Seed or Storage Proteins*
Inset of relevant genes to promote amino-acid production will produce say, maize with high lysine content. In the South African context where we face a protein and essential amino-acid deficit this is important. With high-lysine maize other agronomic difficulties have been encountered in the harvesting and processing of the product.

3. *Nitrogen fixation*
Improving the ability of major crops to fix nitrogen would alone reduce the fertiliser and production costs. However, this remains a long shot as a large

number of genes are involved and a high energy would have to be provided.

Overview

The major danger facing biotechnology today is oversell; time is needed to mature the many promising developments into commercial reality for the farmer.

Despite the economic pitfalls and the unanswered questions, molecular genetics has a useful role to play in tomorrow's agriculture. In order to succeed, a contribution from the scientist, the commercial chemical/seed company and the farming industry is needed in such a way that the limited skills are harnessed to address the various developmental hurdles in an integrated way in Southern Africa.