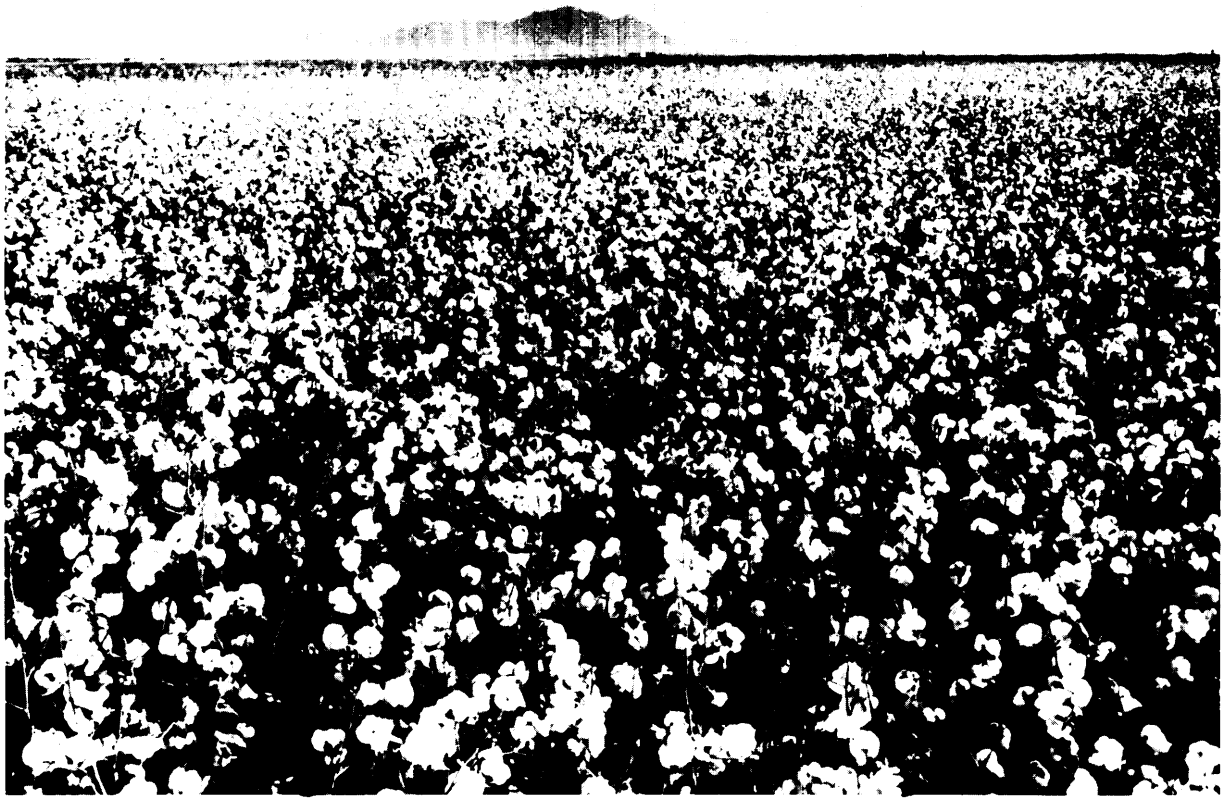
Part I

The Advancing Technologies

Chapter 2

Emerging Plant Technologies



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Each year in the United States weeds, insects, diseases, and poor weather conditions significantly lower crop yields. On average, major crop production in the United States achieves only about 22 percent of the yield theoretically possible under ideal conditions, based on genetic potential. Approximately 69 percent of this loss is due either to unfavorable climate and production using inappropriate farm management practices or poor soils. However, weeds, insects, and disease result in an annual average loss in total yield of 2.6, 2.6, and 4.1 percent respectively (6, 7, 8, 39). Seventy-one percent of crop insurance payments paid in the United States (from 1939 to 1978) were for crop losses caused by drought, excessive water, and cold (6, 8). The financial value of these losses is staggering.

Diseases in fruits, vegetables, grains, and oilseeds result in annual average losses in value of 17, 13, 11 and 13 percent respectively. For some highly perishable fruits, such as raspberries, blackberries, and cherries, losses from disease are estimated to be 38, 34, and 24 percent respectively of their total value. Annual losses in the United States due to viral diseases alone are estimated to be $1.5 to $2.0 billion dollars (5). A recent study estimated that crop diseases resulted in lost revenues equal to approximately 15 percent of the total crop in North Carolina. This value, if extrapolated to the United States as a whole, would result in losses of approximately $12.6 billion per year (8, 28). Loss in value due to weeds has been estimated at 10 to 20 percent of the total crop value; nearly $16 billion per year. Approximately $5 billion is spent annually to control weeds on farms and in rangelands, forests, and waterways (10, 26).

Traditional approaches to managing these problems have included the use of traditional breeding techniques to develop new crop varieties resistant to pests and better adapted to geoclimatic conditions. cultural practices, and the application of chemicals. Pest management is complicated by the fact that plant pests continuously adapt to new management techniques.

The need to develop new approaches to control plant pests is paramount. New pest management methods being developed focus on biological approaches, including the use of biotechnology to alter the plant genome and the use of biological control agents.

Approaches that focus on improving the plant's ability to withstand adversity in general involve genetically modifying the plant to have new characteristics. Scientists genetically modify organisms by altering or adding to an organism's genetic information with the intent to improve the physical characteristics of the organism. The genetic material of living organisms is composed of deoxyribonucleic acid (DNA).[1] The universal nature of genetic material enables scientists to transfer genetic material between species that are normally not sexually compatible, and can be used to modify microorganisms (e. g., bacteria, viruses, and fungi), animals, insects, and plants.

The genetic modification of plants can be accomplished using three different types of techniques: classical, cellular, and molecular (29). The classical methods of genetic modification include those associated with traditional plant breeding. Such methods include:

- fertilization of sexually compatible plants coupled with the preferential selection of those plants containing the desired characteristics,
- the use of chemicals or radiation to mutate the genetic material such that the mutated organism possesses preferred characteristics, and
- traditional cell culturing of plant sex cells such as anthers (the plant organelle that contains pollen) ovules, and embryos.

Cellular techniques involve regenerating a whole plant using culturing techniques, but unlike classical methods, the cellular techniques use tissue cells other than sex cells. Techniques include:

. cell fusion, in which two sexually incompatible plants are hybridized, and
. somaclonal variation,[2] which involves selecting plants that have been regenerated from undifferentiated plant cells—such plants often differ significantly from the parent plants.

[1] The exception to this statement are the viruses whose genetic material is composed of ribonucleic acid (RNA), rather than DNA.

[2] Plants arising from the culturing of undifferentiated cells often differ strikingly from each other and from the parent plant from which the culture was derived. In some unknown way, the process of culturing cells releases a pool of genetic diversity. **Possible explanations of this phenomena include chromosome breakage** and reunion, DNA rearrangement, and point mutations. The amount of variation that occurs is affected by some factors that can be controlled, such as the length of time the cells are cultured, the genotype of the tissue, the medium, and the culture conditions (15. 30).

The molecular techniques include those most commonly associated with biotechnology. Selected genes are isolated and transferred to a host organism using vectors (a piece of DNA that helps to incorporate a new gene into a host organism) or direct transfer techniques such as microinjection, electroporation, or particle guns. Molecular techniques allow for the transfer of selected genes between sexually incompatible species of the same type of organism, or between different types of organisms such as between plants and bacteria.

This chapter will focus on advances made in the use of biological methods to enhance crop production. Emphasis will be given to the use of molecular techniques and the use of biological control agents to enhance both pest resistance and the ability to improve crop production in less-than-ideal conditions.[3]

TOOLS AND TECHNIQUES OF BIOTECHNOLOGY

Biotechnology can be broadly defined as the use of living organisms to alter other organisms. In a practical sense, biotechnology is a set of tools that allow researchers to manipulate genetic material. These tools allow researchers to develop products that could not have been previously produced, and to explore new research questions that significantly expand our scientific knowledge. This section will describe some of the most important tools of biotechnology.

Biotechnology Techniques Used To Create Transgenic Plants

Transgenic crops are those crops whose hereditary DNA has been augmented by the addition of DNA from a source other than parental germplasm, using recombinant DNA techniques. The primary goals of transgenic crop research is to produce crops with improved ability to resist pests (i. e., disease, weeds, and insects); improved ability to grow under less-than-ideal soil and climate conditions; and to improve the quality characteristics of crops (e. g., by changing the oil composition of oilseed crops).

Many advances have been made that improve scientists' ability to create transgenic plants, and several major crops grown in the United States have been successfully transformed (table 2-1). Production of transgenic crops with improved characteristics, however, is constrained by insufficient knowledge of the appropriate genes for transfer; the knowledge base in plant biochemistry and physiology has not kept up with the development of molecular biology and transformation technologies.

To create a transgenic plant, scientists must:

1. isolate and purify the gene to be transferred,
2. find appropriate mechanisms (i.e., vectors or non-vector mechanisms) to transfer the gene into plant cells,
3. attach appropriate regulatory sequences to ensure proper expression of the new gene in the plant,
4. insert proper genetic markers to identify those cells that have been transformed, and
5. regenerate the transgenic cell or tissue into a complete plant.

Advances and methods used to accomplish each step will be described below.

Gene Identification, Isolation, and Purification

Isolating a single gene is complicated by the fact that a DNA sample obtained from a plant usually contains many genes. Researchers must be able to separate the one gene of interest from all of the other genes. Once isolated, the gene of interest is multiplied (cloned) to produce enough genetic material for subsequent uses. The process used to isolate and multiple the gene of interest is generally referred to as shotgun cloning because the process allows for the replication (cloning) of the entire genome (the sum of all genetic information contained in the chromosomes) of the organism.

A sample of DNA is first cut into small pieces, some of which may contain the desired gene. Special enzymes (restriction endonucleases) are used to cut the DNA at specific sites such that each piece has the same types of ends (figure 2-1). Pieces of DNA that have been cut with the same enzyme can be glued together regardless of the source of the DNA. This feature allows, for example, pieces of DNA from plants to be pasted together with DNA pieces from bacteria. It also allows scientists to paste DNA fragments into molecular vectors, pieces of DNA capable of inserting foreign genetic material into a cell. Scientists use vectors to help isolate and purify specific genes. Commonly used vectors include bacterial plasmids (circular pieces of DNA that can be easily in-

[3] Because of the large quantity of research on these technologies. this chapter will cite mainly OTA commissioned background papers and other review articles.

Table 2-1—Transgenic Crops Produced

Grains and oilseeds[a]	Fruits and vegetables	Other
Cotton	Tomato	Alfalfa
Rice	Sugar beet	White clover
Sunflower	Potato	Poplar
Soybean	Peas	Lotus
Rapeseed	Lettuce	Arabidopsis
Corn	Cucumber	Petunia
	Cabbage	Tobacco
	Asparagus	Walnut
	Carrot	
	Pear	
	Celery	

[a]Wheat and barley have not yet been successfully transformed, but it is anticipated that these crops will also be amenable to genetic engineering by the mid-1990s.

SOURCE: Office of Technology Assessment, 1992.

serted into bacterial cells where they can replicate) and bacteriophages (viruses that infect bacteria).[4]

To isolate a gene from an organism, the DNA sample of the organism is cut into many pieces, and all of these pieces are inserted into vectors (e.g., bacterial, plasmid, or bacteriophage). The vectors are then inserted into bacterial cells. As the bacteria reproduce, the vectors containing the pieces of the organism's DNA are also reproduced. This process results in the production of multiple copies of the organism's DNA, which is contained in the vectors. Now scientists have enough copies of genetic material to begin isolating the vectors that contain only the genes of interest. Isolation of the appropriate vectors is accomplished using a probe, a sequence of genetic material that recognizes the desired gene. The probe is used to identify the vectors containing the desired gene. These selected vectors can then be reintroduced into bacteria, where they are replicated many times to produce millions of copies of the desired genes. The desired gene can then be removed from the vector in quantities sufficient to perform subsequent genetic modifications (41).

The above procedure can be easily applied to organisms that possess small genomes, such as bacteria, but is more difficult to apply to more complex organisms such as plants, whose genome size is huge. Additionally, difficulties occur as a result of the lack of knowledge concerning the functions of many plant genes, which precludes the development of probes. Because of these difficulties, additional methods are being developed to improve the isolation of plant genes.

Restriction Fragment Length Polymorphism (RFLP) mapping is used to identify and clone plant genes and to further our understanding of the function of plant genes. RFLP maps take advantage of the fact that corresponding sites in the DNA of individual plants may differ as a result of mutations (referred to as polymorphisms). These polymorphisms can be identified and correlated with known markers (i. e., genes whose function have been identified), which helps to identify the general location of an unidentified gene (2 1). This procedure identifies the approximate location of a specific gene within the plant genome, which limits the amount of plant DNA that must be searched to isolate that specific gene. Once the general location of a specific gene is located, isolating the specific location of the gene depends on other methods still under developments RFLP maps are being made for corn, potato, tomato, rice, bean, pine, soybean, wheat, barley, sorghum, alfalfa, and Arabadopsis (27).

Mechanisms To Transfer Purified Genes Into Plant Cells

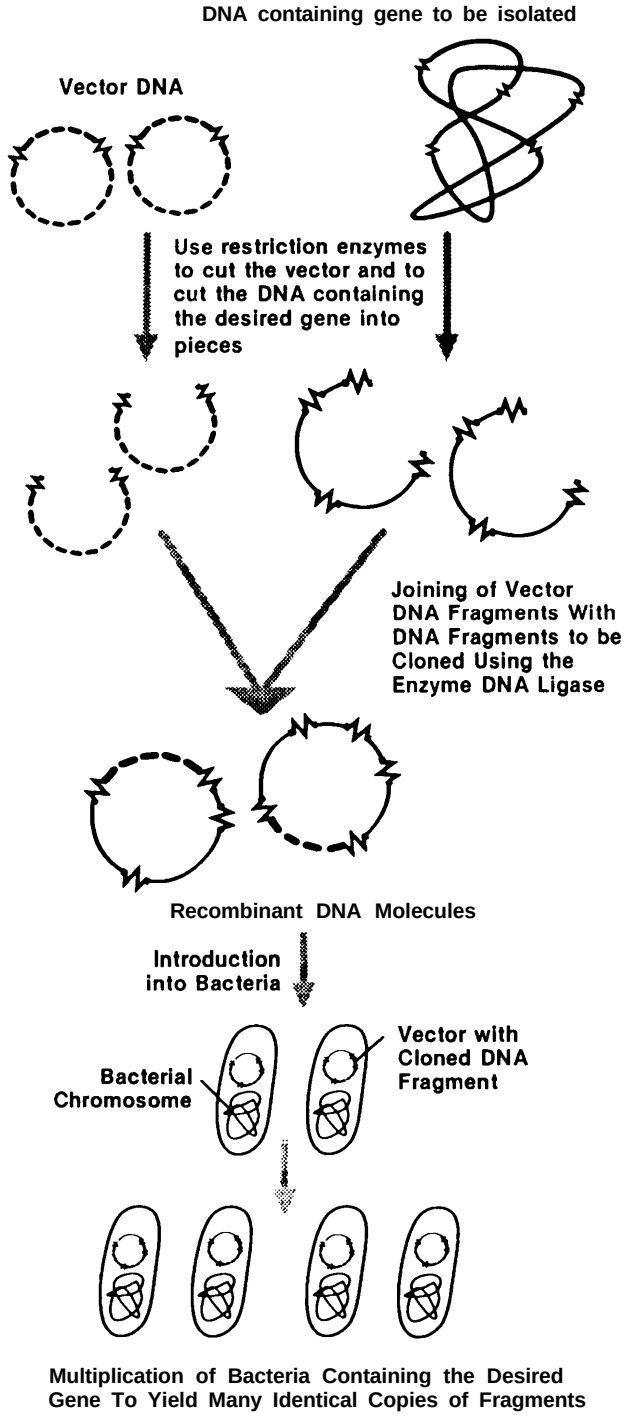
Once a gene has been isolated and purified, it can be transferred to create a transgenic plant. For many dicotyledonous plants (i. e., plants having two seed leaves (cotyledons) and net-veined leaves, such as soybeans), the Ti plasmid of certain strains of the soil bacterium *Agrobacterium tumefaciens is* commonly used as a vector to insert foreign genes into the plant. Unfortunately, Ti plasmids cannot be used to transform monocotyledonous plants (i.e., plants having a single cotyledon and parallel-veined leaves), which includes most of the major cereal crops (e. g., corn, rice, wheat) (27).

Vectorless methods have been developed to transform cereal crops. For example, chemicals (e. g., polyethylene glycol or calcium phosphate) and physical methods (e.g., electrical stimulation) are used to make plant cells leaky so that genetic material can flow in. These approaches have been used successfully to transfer foreign genes into rice and corn (27).

[4] Plasmids are commonly used to construct cDNA libraries (see ch. 3) and bacteriophages are used to construct genomic libraries.

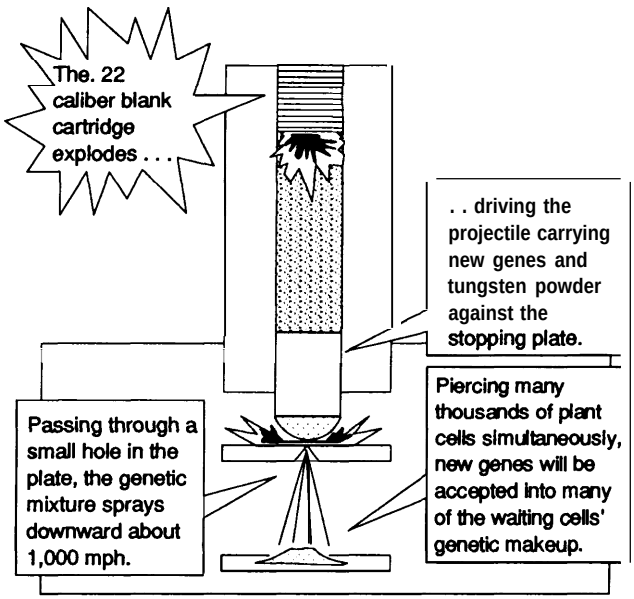
[5] Methods being developed include chromosome walking in which successively smaller overlapping portions of the RFLP fragment are isolated until one ''walks' to the desired gene. This method is constrained by the fact that RFLP fragments may still be too large to clone by the conventional methods described above (27). Another method is called gene tagging, which uses a transposon (a piece of DNA capable of moving around in the genome) to activate the gene of interest. The gene can be located by locating the transposon. Use of this method is inhibited by the size of the plant genome, the lack of transposons for many crops, and the fact that the transposon is often naturally present in multiple copies in crops (27).

Figure 2-1—Identification and Isolation of Desired Gene



SOURCE: Office of Technology Assessment, 1989

Figure 2-2—Gene Transfers With Bioblaster



SOURCE: Agricultural Research Service, U.S. Department of Agriculture

The bolistic method is an alternative vectorless method of gene transfer. This method uses a particle gun to shoot high-velocity microprojectiles coated with DNA into a plant (figure 2-2). It has been used to transfer genes to tobacco, soybean, and corn (27) and can be used to transfer genes to the plant cell nucleus (where the chromosomes are located) and potentially to other cell organelles that contain genetic material, such as the chloroplast (e. g., genes involved in photosynthesis) and the mitochondria (e. g., cytoplasmic male sterility genes used in the development of some hybrid crop varieties).

Currently, there is little control over where the foreign gene is inserted into the host plant. New methods are being developed to target the insertion site, but the frequency of success is low.

Use of Selectable Markers To Identify Transformed Plants

Cells that have foreign genes inserted need to be differentiated from those that have not been transformed. Scientists use markers to identify the transformed cells. The most commonly used marker is the kanamycin resistance gene. Cells containing this gene are resistant to the antibiotic kanamycin and will grow on a culture medium containing high levels of that antibiotic. Untransformed cells not containing the kanamycin resistance gene will not grow on this medium. Genes coding for herbicide tolerance can also be used as a selectable marker to differentiate transformed plants from those that have not been transformed.

Use of Promotors To Control the Expression of the Foreign Gene

Once a foreign gene has been incorporated into the genetic material of a plant, it must still function properly. Scientists use promotors (regulatory genes) to control when and where in the organism the gene is turned on. To date, most transgenic plants contain constitutive promotors, which means that the foreign gene is expressed equally in all tissues and at all development stages. Scientists are trying to isolate promotors that turn the inserted genes on only in specific tissues at certain development stages of the plant, and at a specific time. For example, it is desirable to direct the expression of insect tolerance genes only to the tissues eaten by the insect, such as leaves. The most commonly used plant promotor to date is derived from the cauliflower mosaic virus and is mostly constitutive. However, promotors that respond to light, heat, wounds, and oxygen deficiency, and that show tissue specificity for seeds, pollen, root nodules, and tubers are being identified (27). Understanding the molecular basis of promoter-mediated regulation of gene expression as well as isolation of promoters with varying specificities of expression is critical for the development of new generations of plant-based biotechnology products.

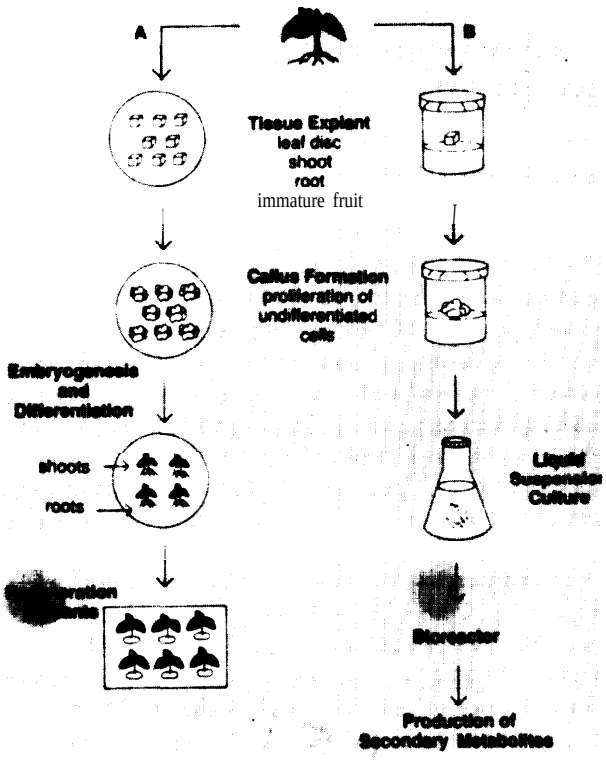
Use of Tissue Culture To Regenerate Transformed Plants

Once a plant cell or tissue has been genetically transformed, it must be regenerated into a complete plant. Advances in plant tissue culturing techniques have now made it possible to regenerate many of the most important crops (figure 2-3).

Early genetic modification research used protoplasm culturing to regenerate the transformed plant cells. Protoplasts are formed by enzymatically removing the outer wall of plant cells. These protoplasts are genetically transformed using the tools of biotechnology, then coaxed into forming a cell wall and eventually growing into a complete plant. However, such regeneration is difficult to achieve with many plant cells, which has lead to the development of callus culturing and cell-suspension methods.

Callus tissue cultures originate from tiny pieces of tissue snipped from seedling shoots or other appropriate plant parts. The tissue is placed in a petri dish containing plant hormones and other plant nutrients. The cells grow

Figure 2-3—Plant Tissue Culture Technology



SOURCE: S.K. Harlander, University of Minnesota

and divide, forming a mound of undifferentiated cells called a callus. When transferred to a regeneration medium, the cells in the callus differentiate into roots and shoots, which then grow into plants. Thousands of plants can be regenerated from one piece of tissue, but the process is labor intensive and expensive.

Methods for the growth of cell suspensions allow for the regeneration of plants from single cells rather than clumps of tissue. Tissues can be agitated in a flask containing a liquid medium, causing the cells to separate. In the appropriate medium, these cells will form somatic embryos that differentiate into entire plants. Embryo suspensions have been used to regenerate wheat, sorghum, and corn (27).

Callus culturing and cell-suspension methods allow for the use of a variety of plant tissues (e. g., leaves, stems, shoot tips, or cotyledons) from many plant species to be used to regenerate new plants. And, *Agrobacterium* particle gun technologies or other direct methods can be used to transform these tissues. Thus, most major crops can now be genetically engineered and regenerated to complete plants.

Other Biotechnology Techniques

Biotechnology is most closely identified with the use of recombinant DNA technologies to produce transgenic crops as described above. However, other technologies, some of which also involve the use of recombinant DNA, will also play a significant role in the development of new plant technologies. Some of these technologies are described below.

Antisense Technology

Antisense technology is a powerful research tool that enables scientists to study the physiology and development of organisms. It is also useful in the production of transgenic crops that have new characteristics (37). For example, this technology is being used to prevent softening in tomatoes (see *Biotechnology in Food Processing).* The power of the technique lies in its ability to eliminate or reduce the expression of a gene in an organism.

An analogy that might help to explain how this technology works is to view the expression of a gene as being similar to reading a sentence. For the sentence to make sense, it must be read in a certain direction; sentences that are read backwards, for instance, don't make sense. Gene expression is similar, A gene must be read in a certain direction to produce a gene product that makes sense to the organism (i. e., it is a functional compound).

The antisense technology consists of incorporating into an organism a synthetic gene that reads backwards (i. e., a product is made that doesn't make sense to the organism). The expression product of this backward-reading gene is a mirror image of the expression product of the same gene when it is read forward. When the expression products of the forward and backward genes meet,[6] they stick together, thus inactivating the product of the forward-reading gene (figure 2-4). Thus, the antisense technology can be used to inactivate selected genes in the plant. Use of the technique, however, is constrained by the need to know the precise nucleic acid sequence of at

[6]Technically, when a gene is expressed, it is first copied and modified to a second compound called messenger ribonucleic acid (mRNA). The mRNA then serves as the template for the subsequent production of proteins. It is the mRNA, rather than the protein, that meets and causes the inactivation.

Photo credit: U.S. Department of Agriculture, Agricultural Research Service.

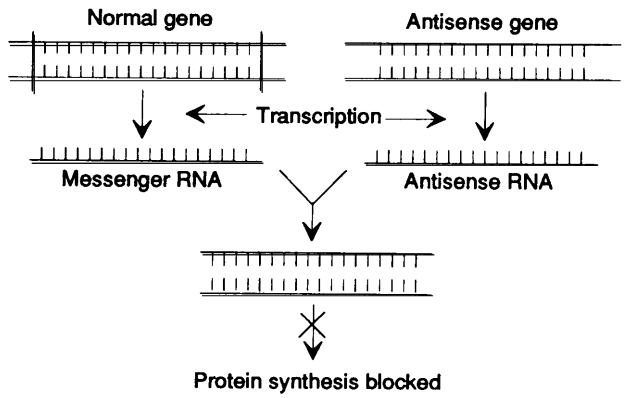
Molecular biologist at UC/USDA Plant Gene Expression Center successfully transferred new genes into cells of corn using a gene gun.

least a portion of the gene that codes for the expression product to be inhibited.

Polymerase Chain Reaction

The Polymerase Chain Reaction (PCR) technology enables scientists to rapidly generate large amounts of genetic material from a trace amount, which would otherwise be too small to analyze. PCR is an enzymatic process carried out in repeated cycles, each of which doubles the amount of DNA present. Small flanking sequences of DNA are identified on each end of the DNA sequence that is amplified. These flanking sequences are then used to create complementary strands of DNA that serve as primers. These primers are then annealed to the flanking sequences, and when appropriate enzymes and nucleic acids are added under the proper conditions, a new DNA strand is formed beginning at the primer and extending across the sequence of DNA to be replicated, such that a copy of this sequence is made. This methodology is rapid, sensitive, and relatively easy to carry out; about 25 cycles can be carried out in an hour. PCR reduces the difficulty of isolating and manipulating specific DNA

Figure 2-4—Antisense Technology



SOURCE: *Science* 253:510, 1991, p. 510.

sequences, and makes it possible to study biological problems related to very small amounts of genetic material (2).

Monoclonal Antibodies

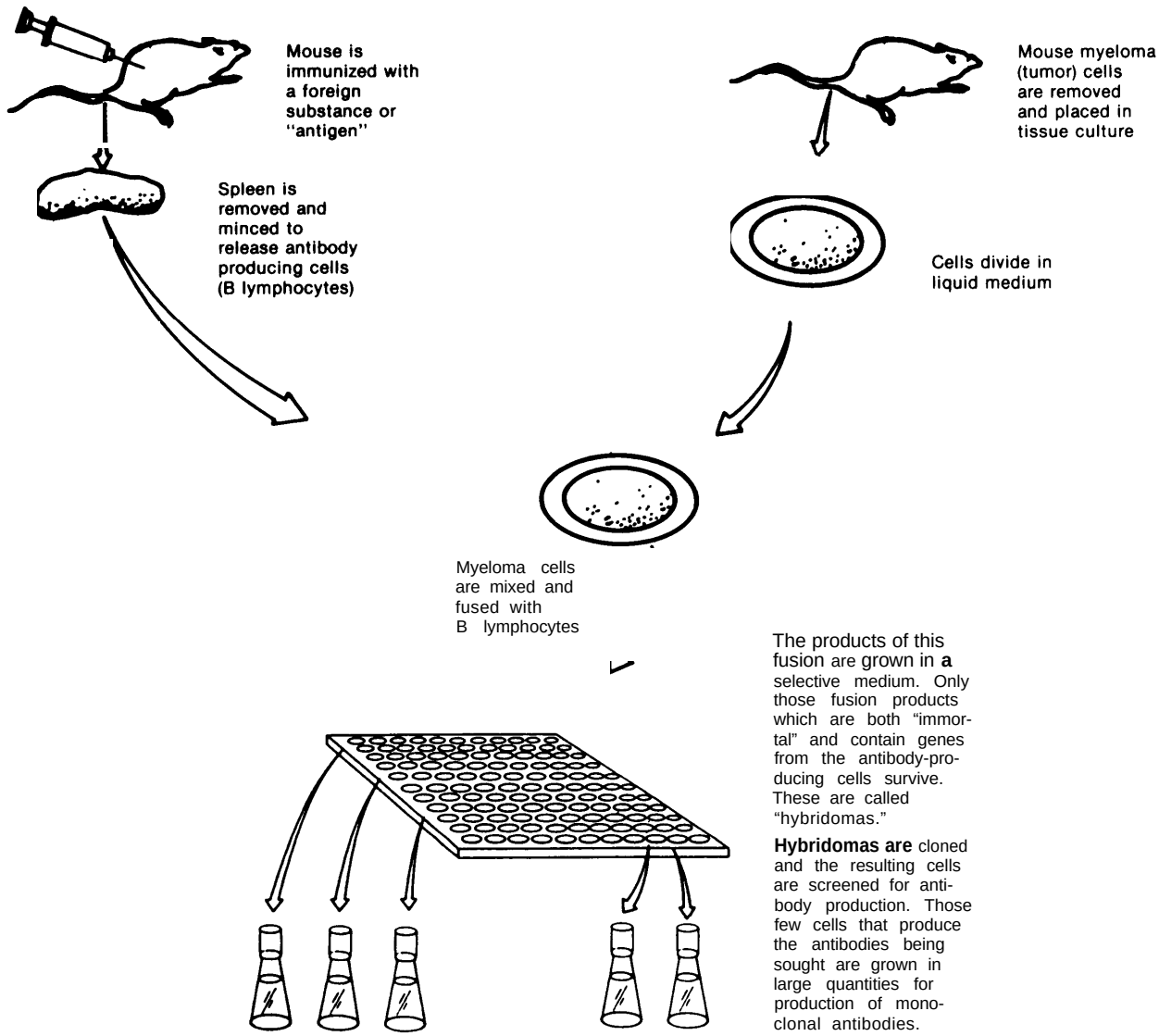
Monoclonal antibodies are identical antibodies that recognize a single, specific antigen (substance that elicits an immune response) and are produced by a clone of specialized cells. Their uses include the detection of residues and toxins in food, and as animal vaccines.

Monoclonal antibodies are produced by immunizing a donor animal (usually a mouse) with a target substance (figure 2-5). The animal's spleen is removed and dissociated into single-cell suspensions. These cells, some of which produce antibodies to the target substance, are removed to a nutrient medium. Spleen cells can survive only a few days in this medium, so to increase their life expectancy, the spleen cells are physically fused to a tumor cell, which can live indefinitely in tissue culture medium.[7] This fusion produces a hybrid cell containing the combined genetic information of both parental cells, which is capable of secreting the antibody produced by parental spleen cell and, like the parent tumor cell, can live indefinitely in culture medium.

This hybrid cell (called a hybridoma) can be isolated, cloned to ensure purity, and grown in mass culture where the secreted antibody accumulates in the culture medium.[8] The antibody (called a monoclonal antibody) that accumulates consists of a single antibody type rather than a mixture of antibody types (as occurs with traditional

[7] The spleen cells are fused in the presence of an agent, such a polyethylene glycol, to myeloma cells—tumors of B lymphocyte origin.
[8] Alternatively the hybrid cells can be grown as tumors in the peritoneal cavities of mice where very high levels of antibody accumulate in the ascites fluid surrounding the tumor.

Figure 2-5—Preparation of Monoclinal Antibodies



SOURCE: Office of Technology Assessment, 1988.

antibody production methods). It is this purity that makes monoclinal antibodies so useful.

Application of Biotechnology Techniques To Create Transgenic Plants

The tools of biotechnology are allowing researchers to explore new means to control plant diseases, insect pests, and weeds. Tissue culturing and genetic engineering, combined with traditional agricultural research methods, are allowing scientists to alter plants or biological control agents to achieve enhanced efficacy and host range in controlling plant pests. Biotechnology is also being used to improve a plant's ability to withstand environmental stresses, such as cold, drought, and frost, improve the shelf-life of fruits and vegetables and is being used to develop value-added products from agricultural commodities (e. g., increased carbohydrates. modified oils, and proteins that contain essential amino acids). In addition to developing new products, the tools of biotech-

Photo credit: U.S. Department of Agriculture, Agricultural Research Service.

Plant molecular biologist examines successful results of the cloning of a gene necessary for plants to synthesize ethylene, the ripening hormone. More recently, scientists have blocked this gene, producing genetically engineered tomatoes that ripen on demand.

Photo credit: Monsanto Co.

Tomato plants that show one stripped by caterpillars and one not. The plant not stripped contains the *Bacillus thuringiensis* toxin gene.

nology are expanding the knowledge base of plant resistance and the interactions of plants, pests, and biological control agents with the rest of the ecosystem.

Genetic Engineering of Plants for Insect Control

Traditional breeding programs have successfully produced varieties of alfalfa, cotton, corn, rice, sorghum, soybean, and wheat that have been resistant to, or tolerant of, key pests and will continue to play an important role in developing insect resistant plants for some time in the future. However, the tools of biotechnology have created the possibility of selectively engineering plants for insect resistance. Biotechnology will permit the transfer of resistance genes into plant species for which the resistance gene is not inherent. Biotechnology is also being used to improve the understanding of mechanisms by which plants are resistant to insects.

Few genes known to produce insecticidal proteins have been identified. Candidate genes must code for proteins that are stable in the plant cell, are not rapidly digested when consumed by insects, have high activity against feeding target insects, and are safe for nontarget invertebrates and animals. Insecticidal proteins produced by the spore-forming bacteria *Bacillus thuringiensis* (Bt) are among the few known to meet these criteria,

The Bt bacteria produces crystals that contain compounds toxic to insects. Insects feeding on plants contaminated with Bt bacteria ingest the crystals, which are dissolved in the insect midgut, releasing the protein toxins. Different strains of the Bt bacteria produce insecticidal toxins specific to Lepidoptera (butterflies and moths) only, to Diptera (flies and mosquitoes) only, to Coleoptera (beetles) only, and to both Lepidoptera and Diptera.

Genetic engineering is being used to improve the delivery of the Bt toxin to insect pests by incorporating the insecticidal gene into other vectors (see Biological Control of Anthropoids: Pathogens) or by transferring the insecticidal gene directly to plants. Genes coding for the Bt insecticidal protein have been cloned and inserted into tobacco, tomato, and cotton plants among others (1). Transgenic plants producing Bt insecticide are expected to be commercially available by the mid to late 1990s.

Genes for some insect trypsin inhibitors have also been cloned. Trypsin inhibitors are compounds that, when present in large amounts, may reduce the ability of an insect to digest plant material. Some plants, such as the seeds of cowpeas and beans, contain large quantities of trypsin inhibitors (i.e., 1 to 2 percent of the total protein), and the levels in plant leaves may be increased in response to mechanical damage or insect feeding. Trypsin inhibitor genes derived from tomatoes have successfully controlled the growth of insect larvae when transferred to tobacco plants. Transgenic plants genetically engineered to produce trypsin inhibitors may be available by the end of the decade (1).

Genes that code for lectins and for arcelin are also potential candidates to confer insect resistance to transgenic crops. Lectins are sugar-binding proteins found in the seeds of peas and common beans. They are effective against bean weevils and cabbage weevils. Arcelin is

produced in the seeds of wild beans and is toxic to bean bruchid pests (1).

Genes coding for insecticidal proteins other than Bt toxins and trypsin inhibitors must be identified. RFLP maps are being used in tomatoes, for example, to discover the location of insect resistance genes in plants. The development of tissue-specific promotor sequences and promotors that respond to selected environmental stimuli are needed to improve the efficacy of insect control.

Genetic Engineering of Plants for Weed Control

The presence of weeds in crops decreases productivity and crop quality. To control weeds, farmers commonly apply herbicides. Most herbicides act by inhibiting key enzymes in photosynthesis or other essential plant biosynthetic pathways. Plant species respond differently to herbicides depending on the sensitivity of plant enzymes to the herbicide or the ability of the plant to metabolically inactivate the herbicide. These abilities explain why herbicides are often effective against either grassy or broadleaf plants, but not both (26).

Herbicide manufacturers would like to develop broad-spectrum herbicides active against all economically important weeds, but their efforts have be constrained because broad-spectrum herbicides not only kill weeds, but they injure crops as well. Two approaches have been taken to minimize crop damage when using broad-spectrum herbicides. One approach is to use herbicide antidotes, compounds that enhance the metabolic inactivation of herbicides in plants (19, 20). Few such antidotes have been discovered, however, and it is unlikely that this approach will yield significant success in the near future. The alternative approach is to develop crop varieties that are resistant to the herbicide used.

Traditional methods have been used successfully to develop herbicide-tolerant crops. Tissue culture and plant regeneration techniques have produced tobacco and soybean varieties tolerant to sulfonylurea herbicides and corn varieties tolerant of imidazolinone. Attempts to develop herbicide-tolerant crops using tissue-culture techniques are most successful when the herbicide affects only one compound in a plant biosynthetic pathway (i. e., it has a single target site) and a mutation in that compound confers herbicide tolerance without affecting the growth of the plant, or when the mutation of a single plant gene increases the ability of the plant to inactive the herbicide or to absorb less of the herbicide. Use of these methods is constrained by the lack of naturally occurring herbicide tolerance genes in crops (26).

Genetic engineering techniques overcome the lack of naturally occurring herbicide resistance genes in plants by allowing for the transfer of these genes between crop species. Thus, crops tolerant to a specific herbicide (but not all herbicides) can be developed. Three different approaches have been taken to engineer crops successfully for herbicide tolerance, the first of which are expected to be commercially available by the mid 1990s (table 2-2). One approach relies on making the crop produce excess quantities of the enzyme normally affected by the herbicide. By producing an excess quantity of the enzyme, a sufficient quantity is still available to catalyze important plant biosynthetic pathways even though some of the enzyme has been inactivated by the herbicide. Excess production can be achieved by inserting several copies of the gene coding for the enzyme into the plant, or by using promotor sequences that cause excessive expression of the genes coding for the enzyme, This method has been used successfully to produce crops tolerant to glyphosate and phosphinothricin (26).

The most commonly used approach to produce crops tolerant to herbicides is to alter the gene coding for the enzyme affected by the herbicide in such a way that the resulting altered enzyme is still effective in the plant, but is not inactivated by the herbicide. This altered gene is then inserted into the plant where it produces an altered enzyme that confers herbicide tolerance. This approach has been used to produce crops tolerant to glyphosate, sulfonylureas, phosphinorthricin, atrazine, and imidazolinone.

The third approach is to transfer to plants those genes that code for enzymes that inactivate herbicides. This approach has been taken to confer plant tolerance to bromoxynil, 2,4-D, and phophinothricin.

An alternative approach to weed control is to develop crops that produce their own herbicides. These plant-produced herbicides, called allelochemicals, can be either volatile organic compounds released into the air or soil where they can be absorbed by the weed or nonvolatile organic compounds released as root exudates or leachates of other organs, such as seeds. Most volatile allelochemicals are terpenoids whose secretion increases with rising temperatures, while most nonvolatile allelochemicals are aromatic chemicals (26). Significant research is still needed before crops can be engineered to produce allelochemicals. Alternatively, it may be possible to identify and use plants known to naturally produce allelochemicals as cover crops or in low tillage

Table 2-2—Current Targets for Crop Modification for Herbicide Tolerance

Herbicide	Research institution	Commercial introduction	Weed/crop targets
Atrazine	Ciba Geigy, Inc	Not expected to be commercialized	NA
Bromoxynil	Calgene, Rhone-Poulenc	Mid 1990s	Broadleaf/dicots
Betanal	Schering	Late 1990s	Broadleaf/sugar beet
2,4-D	Max Planck	Not a commercial target	NA
Dicamba	Sandoz	Late 1990s	Broadleaf/NA
Glyphosate	Monanto, Calgene	Mid 1990s	Broad spectrum soybean, rape, cotton, corn
Imazapyr	American Cyanamid Molecular Genetics	Early to mid 1990s	Broad spectrum/corn
Metribuzin	Mobay	Late 1990s	Broad spectrum/ soybean
Basta	Hoechst	Mid 1990s	Broad spectrum/ rape, beet, potato, soybean, corn
Sulfonyl ureas	DuPont	Mid 1990s	Broad spectrum/ soybean, rape

NA = Not applicable.

SOURCE: Off Ice of Technology Assessment, 1992

situations to control weeds. For example, it has been shown that certain cucumber strains produce compounds toxic to the weeds proso millet and barnyard grass under field conditions. The possibility of using alleochemical-producing plants is also being explored in fruit production (33).

Understanding the nature of allelochemicals in addition to the advances that have been made in elucidating the mechanisms of herbicide action is expected to enhance the design of future herbicides.

Genetic Engineering of Plants for Disease Control

Bacteria, fungi, parasitic seed plants, nematodes, insects, and viruses, among other organisms, can destructively alter the structure or physiological processes of plants, resulting in disease. However, plants possess the ability to resist the invasion of pathogenic organisms. All of the plants of a species can be resistant to a pathogen, or certain varieties of a plant species can be resistant to a subspecies of the pathogen (i. e., cultivar specificity). The interaction of bacterial and fungal pathogens with plants is helping to elucidate the mechanisms by which plants resist pathogenic organisms **(27).**

The ability of plants to resist pathogenic organisms involves the complex interaction of genes in both the plant and the pathogen. The interaction of compounds produced by plant resistance genes and genes in the pathogen (i. e., avirulence genes) triggers a hypersensitive response. Plant cells initially infected by the pathogen die, preventing the spread of the pathogen to the rest of the plant. Thus, the pathogenic effects remain localized at the site of initial infection, and disease is prevented from spreading throughout the plant.

The mechanisms by which pathogens infect plants are also being elucidated. Pathogenic microorganisms contain pathogenicity genes that produce compounds toxic to the plant and/or allow the pathogen to attach to the plant, penetrate the cuticle and degrade the walls of plant cells, and degrade chemicals produced by the plant in its own defense. These pathogenicity genes can be activated by signals from the plant itself. For example, the presence of cell wall degradation products in plants can trigger the production of enzymes in some pathogenic fungi that degrade the cell wall. In a similar manner, compounds produced by pathogens trigger a response by the plant to the pathogen. Plant defense genes are stimulated to produce compounds that may be toxic to pathogens, reinforce the cell wall, and/or inhibit enzymes produced by the pathogen (27).

Efforts are underway to clone and characterize pathogen and plant genes involved with resistance. To date, no plant resistance genes have been cloned, however, avirulence genes from bacteria and viruses but not fungi, have been. Additionally, few plant defense genes have been identified and cloned. Only the gene coding for chitinase, a compound that is toxic to fungi, has been shown to confer disease resistance when transferred to tobacco. Also a compound derived from moths, when

Photo credit: Richard Nelson, Samual Roberts Noble Foundation.

Transgenic tomato plant expressing the coat protein gene of tobacco mosaic virus (left) and control plant (right).

transferred to tobacco, decreased the severity of an infection by the bacteria *Pseudomonas solanacearum.* Given the **state** of the art, it is highly unlikely that plants resistant to bacteria and fungi will be developed before the year 2000 (27).

Greater success has been achieved in developing plants resistant to viruses. Plants have long been known to display cross protection, a phenomena that occurs when plants infected with a mild strain of a virus do not develop severe symptoms when challenged with a stronger strain of the same virus. Cross protection is comparable to immunity in animals, although plants do not have immune systems and the mechanism of protection differs. Although cross protection has been achieved in plants by inoculating individual plants with a mild virus strain, this process is very labor intensive and carries a small risk that the virus strain used will become more virulent and act in a synergistic fashion with other viruses (27).

Genetic engineering has been used to avoid these problems. Genes coding for virus coat proteins (i.e., the proteins that make up the shell that surrounds viruses), other

Table 2-3—Virus Coat Proteins Engineered Into Plants

Alfalfa mosaic virus
Cucumber mosaic virus
Potato viruses S, Y, and X
Potato leaf roll virus
Tobacco mosaic virus
Tomato mosaic virus
Tobacco rattle virus
Tobacco streak virus
Soybean mosaic virus
Papaya ringspot virus
Tomato spotted wilt virus

SOURCE: Office of Technology Assessment, 1992.

virus proteins, and virus RNA sequences can be introduced into plants to elicit a resistance response (3, 4). Plants engineered with coat protein genes from a specific virus have resisted subsequent infection by the same virus, and in some cases to related viruses having similar coat proteins. Currently, many viral coat protein genes from different plant viruses have been transferred to plants to confer resistance (table 2-3) (4). The mechanism by which protection occurs is not fully understood. Most evidence suggests that the accumulation of viral coat proteins in plant cells interferes with the release of viral RNA needed to initiate infection (4).

In addition to viral coat proteins, other viral genes have been transferred to plants. Those having potential for virus control include: genes for virus replication, antisense RNA, satellite RNA, and ribozymes. The antisense technology has also been used to inhibit viruses in plants. Other approaches include transferring satellite RNA sequences (small RNA sequences that depend on helper viruses to replicate and package new virus particles) to plants where they have protected the plant from developing symptoms in response to an infection by the helper virus. Genes coding for RNA sequences that act like enzymes (i.e., ribozymes) have also been transferred to plants where they have cleaved invading viruses (27).

Genetically engineered dicotyledonous plants resistant to certain viruses are expected to be commercially available by the mid 1990s. Monocotyledonous plants resistant to viruses will probably not be available until the late 1990s or early the next century. Currently, only a few genes with potential for controlling fungi and bacteria have been identified, cloned, and introduced into plants (see table 2-4).

Genetic Engineering of Plants for Thermal and Water Stress Tolerance

Progress in improving the tolerance of plants to water and thermal stress will depend, in part, on better ways

Table 2-4—Disease Resistance Genes Introduced Into Plants

Disease pathogen	Gene/plant
Fungal	Chitinase/tobacco
Bacteria	Antibacterial protein from moth/ tobacco, potato
	Enzyme to detoxify bacterial toxin
Viral	Viral coat protein
	Other virus genes
	Satellite RNA
	RNA enzyme (ribozyme)
	Antisense RNA

SOURCE: Office of Technology Assessment, 1992

of defining and quantifying these stresses as well as nonstress states. Defining these stresses is further complicated by the fact that water stress and temperature stress are not easily separated, particularly at high temperatures. New tools, such as remote and contact sensing,[9] are being developed to detect plant stress (9).

The lack of detailed knowledge of the physiology of water and temperature stress tolerance also constrains progress in this field. The root system of the plant exerts major control over water uptake. Little research has been conducted to measure root response to water and thermal stress. Most measurement techniques used to date are disruptive if not destructive to root systems. New techniques are needed to determine factors that affect the distribution of roots in the soil and the ability of the roots to absorb water and transport that water through the vascular tissues of the plant (9).

Plant-cell culturing, combined with selection for enhanced ability to adjust the salt and water concentration of plant cells (osmotic pressure), has been shown to be effective in improving drought tolerance. However, while improved sensitivity to osmotic pressure has increased the survival of the plant, it does so at the expense of plant growth and yields (34).

Some plants contain genes that code for proteins conferring tolerance to extremes of temperature or drought; these genes are possible candidates for isolation and transfer to other plants through genetic engineering techniques. For example, tobacco cells that are exposed to gradually higher levels of salt synthesize several novel proteins. One such protein is osmotin, whose synthesis is regulated by several mechanisms, including exposure to low water environments or changes in endogenous levels of the hormone abscisic acid (ABA). ABA is known to lower the rate of transpiration from leaves and prevent water loss. The role of osmotin in cellular osmoregulation is now under investigation (9).

Some plants, when challenged by elevated temperatures, produce heat shock proteins. Genes coding for several of these proteins have been sequenced and their promoter regions identified. However, the metabolic functions of most of these proteins are not understood, and this constrains their use in biotechnology to improve plant tolerance to elevated temperatures (9).

In general, the fundamental research needed to understand the mechanisms of tolerance to thermal and water stress simply has not kept pace with the development of biotechnology tools, and thus, scientists do not currently know what genes to transfer into plants to improve tolerance for these stresses. Thus, genetically engineered plants tolerant to elevated thermal or water stress are unlikely to be developed within this decade. However, antifreeze proteins have been transferred to plants and production of plants with improved cold tolerance may become available within 10 to 15 years. Plants transgenic for antifreeze proteins have the potential to improve cold hardiness by lowering the temperature at which leaves freeze (12, 17). Antifreeze proteins from fish are also being used to improve the post-harvest freezing and thawing qualities of fruits and vegetables by inhibiting ice recrystallization in tissues (22).

Biotechnology in the Food Processing Industry

Historically, the food processing industry has had to accept and adapt to heterogeneous raw materials. Biotechnology can be used to better tailor food crops to meet food processing and consumer needs. Tissue-culture techniques are being used to select or construct crop varieties with improved functional, processing, or nutritional characteristics (table 2-5).

Plant tissue-culture techniques can be used to produce food flavor and coloring ingredients. These methods could potentially replace production and extraction of these ingredients from plants (15, 18). For example, a private company recently has succeeded in using tissue culture techniques to produce vanilla (14).

[9]Contact sensing requires contact with plant tissues and may require destruction of at least part of the plant. It involves the direct determination of the state of a physical, biological, or chemical quantity. Remote sensing quantitates parameters measured by using a sensor to detect electromagnetic waves emitted or reflected by plants.

Photo credit: U.S. Department of Agriculture, Agricultural Research Service.

Framed by drought-dried cornstalks, drought-resistant lima beans stand tall and lush in test plot. Scientists hope that genetic engineering researchers can isolate the genes that give the lima bean such a high degree of drought tolerance.

Table 2-5—Use of Tissue Culture To Improve Food Characteristics

Crop	Characteristic
Tomato	Increased solids
	Increased shelf life
Carrots	Increased sweetness, crunchiness
Celery	Decreased stringiness
Corn	Improved amino acid composition
Rapeseed	Decreased saturated fatty acids

SOURCE: Office of Technology Assessment, 1992.

Genetic engineering is also a means of altering food characteristics. Genes coding for enzymes involved in starch and lipid biosynthesis are being isolated and cloned, enhancing the prospects of engineering plants with specific composition of starch and oil. Genes coding for floral pigment pathways are also being isolated. Plants potentially can be engineered to produce pharmaceuticals such as blood clotting factors and growth hormones. For example, oilseed rapeseed has been genetically engineered to produce enkephalins (40). In addition, antisense technology is being used to eliminate toxins allergenic compounds, or off-flavor components in plants, and to delay ripening of tomatoes (15).

Photo credit: DNA Plant Technologies, Inc.

Vegi Snax is an example of successful application of plant tissue culture for selection of crop varieties with improved functional, processing, and nutritional characteristics.

Biotechnology is also being used to improve microorganisms used as vegetable starter cultures and in brewing and baking (i.e., organisms used in making sauerkraut, pickles, olives, soysauce, wine, beer, and bread) such that these organisms tolerate different temperature and pH ranges. Similar work is being conducted with microorganisms used to produce food ingredients such as acetic acid, citric acid, niasin, vitamin B 12, xantham gum, and monosodium glutamate. In addition, genetically engineered enzymes are being developed to treat food processing wastes (18).

Finally, biotechnology is being used to develop methods to assay levels of pathogens, toxins, and chemical contaminants in raw ingredients and final products. DNA probes and poly and monoclinal antibody kits are beginning to replace traditional bioassay methods. For example, many of the assay procedures used to detect pesticide residues in food are monoclinal antibody kits (18).

THE TOOLS AND TECHNIQUES OF BIOLOGICAL CONTROL

Approaches Used in Biological Control

Biological control of pests relies on using living natural enemies (e. g., parasites, predators, and pathogens) to reduce pest populations to levels lower than would otherwise

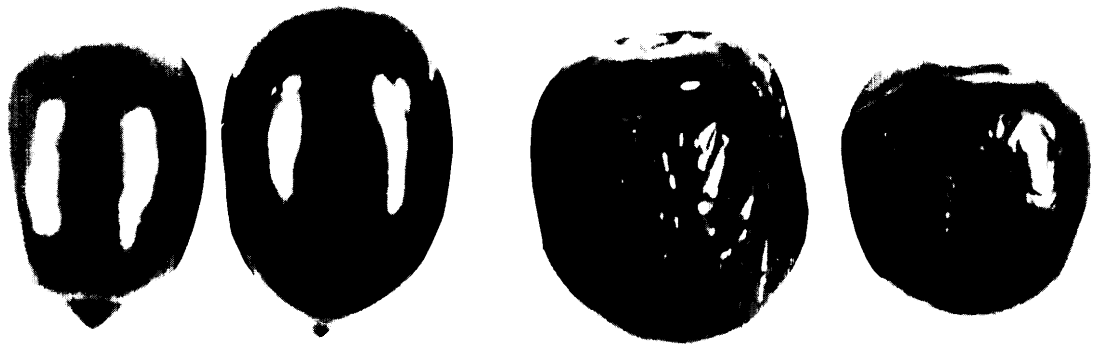


Photo credit: Calgene, Inc.

Antisense tomatoes (left) and control (right) 3 weeks after harvest.

occur (13). Parasitic organisms are those whose development takes place in or on a single host organism; predator organisms are those that consume other organisms as a food source; and pathogenic organisms are those that cause disease in other organisms. Many organisms, including insects and other arthropods (e. g., spiders and mites), bacteria (and related organisms such as rickettsiae and mycopiasmas), viruses, fungi, protozoa, and nematodes are being used as biological control agents to manage weeds, insects, and other arthropod pests, as well as disease organisms in economically important plant species. Biological control methods have been used in the United States on a limited basis for at least 100 years. Approaches used can be classified into three common types-the classical approach, augmentation, and conservation (25).

Biological control agents used to control nonindigenous pests, particularly those introduced from other countries, is called the classical approach. When a non-native pest is introduced into a new environment, often there are no natural enemies to control that pest. The classical approach searches the area of origin of the pest and identifies natural enemies. These natural enemies are then introduced into the new environment to control the pest (25). Attempts are made to establish the introduced natural enemies as part of the ecosystem so that pest suppression will be permanent.

The augmentation approach focuses on increasing the existing population of indigenous pest enemies. Small numbers of natural enemies can be released periodically, as needed, to increase the indigenous population to levels sufficient to control pest numbers at levels below those that cause serious economic problems. The newly released natural enemies are expected to become part of the ecosystem, and to help suppress more than one generation of pests (25). This approach is similar to administering a booster shot to augment indigenous-pest enemy populations.

Alternatively, large numbers of natural enemies can be released at one time with the intent of quickly suppressing the pest population by creating an epidemic-like situation. The control agent (i. e., natural enemy) is not expected to become a permanent part of the ecosystem and the natural enemy is not expected to control more than one generation of the pest. The natural organisms used with this approach are **usually microorganisms,** such as bacteria and fungi. They are manufactured, formulated, standardized, packaged, registered as pesticides, and applied to pests using methods and tools similar to those used for chemical pesticides. **Because** of these similarities to chemical pesticides, this strategy is often referred to as the microbial pesticide or inundative approach to augmentation. This approach generally requires regular application because the control agents do not survive between crop seasons. or survive in insufficient number to be effective the next season, or are prevented by other factors from causing significant disease in the pest population (10, 16).

Conservation practices can be used to protect and maintain natural enemy populations by manipulating the environment. such as altering cropping patterns and farm management practices to enhance the indigenous population, maintaining refuges and providing feeding and nesting sites for natural enemies, and by applying pes-

ticides only when pest populations exceed specified levels (16, 25, 35).

In general, the classical method of biological control has been the approach most frequently and successfully used to control weeds, insects, and other arthropods in the United States. This is perhaps not surprising given the large number of pests that are of foreign origin. For example, an estimated 39 percent of the 600 most important arthropod pests in the United States are of foreign origin and more than 630 additional foreign arthropods are on the list of lesser pests (36). Based on past history, it is predicted that exotic arthropod species will continue to be added at a rate of about 11 species per year and that approximately 7 of those species will become significant pests. Clearly the classical approach will continue to be a major biological control methodology.

Biological control approaches have had limited success against pests in grain and row crops.[10] Biological control has been most successful against naturalized permanent pests in areas of low disturbance (such as rangeland, pastures, forests, and some aquatic habitats) where the targeted pest is the dominant species, and where the end goal is a stable plant community. The poor record of success in grain and row crops is often attributed to the fact that grain crops only persist for short periods of time, during which the natural enemy must discover the crop and become established, must find and attack its host pest, and must increase its population to numbers sufficient to reduce the pest population significantly. The abrupt end of the crop season precludes the establishment of stable interactions between pests and natural enemies in grain crops (13, 16, 25).

It is perhaps for these reasons that the microbial pesticide approach using fast-acting pathogens has received more research attention than any other biological control approach to pest suppression in grain and row crops. The bacterium *Bacillus thuringiensis*, which produces compounds that are quickly toxic to some insects, can be used effectively in this manner. The microbial pesticide approach is also being taken to develop fungi that control weeds (10, 16).

The conservation approach has received the least research attention. Little incentive exists for the private sector to develop these technologies because the product that is developed is management information. Successful development of this approach will most likely fall to public sector researchers. Methods to control communities of organisms in a systemic fashion rather than a single control agent are needed (11).

Research Needs

Extensive research in many disciplines will be required if biological control is to become more widely used. A better fundamental understanding of pest-natural enemy interactions, ecology, and population biology is needed, as well as attention to more applied problems of mass rearing, formulation, and delivery required to make these control agents commercially viable. Successful development will require a multidisciplinary approach and will draw from expertise in many fields, including: systematic (taxonomy), ecology, behavioral science, physiology, genetics, chemistry, and epizootiology (the study of population disease at the population level), among others (10, 16, 25, 38).

Taxonomic, biochemical. and genetic comparisons of pests from the same or similar species taken from geographic areas of suspected evolutionary origin also are needed. These studies can help identify pests and their natural enemies, improve understanding of the relationship between pest and enemy, and determine the geographic distribution of each. Use of classical biological control methods will be enhanced if techniques can be developed to detect and eliminate parasites and pathogens from the imported natural enemy cultures (10, 16, 25, 38).

An improved understanding of the natural enemy-pest dynamics and factors that enhance the effectiveness of control is needed. Elucidation of the structure and roles of insect hormones and compounds that attract or repel pests is needed. Additional research is needed to understand the natural enemy population (i. e., infectivity, virulence, specificity of host; biological fitness including survival, persistence, and dispersal; the role of population density, etc.), the pest population (i.e., susceptibility, development of resistance, mechanisms of immunity, population density impacts, and distribution), the effects of the abiotic and biotic environment (i. e., weather, soils, host plants, biotic transport agents, sunlight, cropping patterns, etc.), and the environmental impacts of releasing predators, parasites, and pathogens to control pests (10, 16, 25, 38).

A major constraint to using the augmentation approach to biological control is the inability to cost-effectively

[10] Recent work With baculoviruses to control insects has been promising and this biological control agent may prove to be an exception to this statement (16).

raise large numbers of parasites, predators, and pathogens. The life cycles of many natural enemies are complex and raising these organisms in an artificial setting is difficult. New mass rearing techniques need to be developed for many biological control agents.

For natural enemies that are parasitic insects, laboratory rearing requires maintaining not only the host insect, but the food source of the host insect as well, which may include plants that are themselves difficult to grow. Thus, mass rearing of a parasitic insect requires maintaining both an appropriate plant population and host insect population, a costly arrangement that points to the need to develop artificial diets (10, 25).

Viruses can also be difficult to mass produce. Viruses are obligate cellular parasites and must be produced within living cells. For viruses that are pathogenic to insects, this can be accomplished either by infecting whole insects or by infecting cultures of continuous cell lines derived from the host insect. Recent advances in insect cell culture is improving the prospects of virus pesticide production. Significantly, most of these advances are being made in the biomedical field rather than the agricultural field, because biomedical industries are using certain classes of viruses (such as baculoviruses) as vectors to express foreign genes for high-level production of biological and pharmaceutical products (16).

Mass production techniques for fungal spores are also needed. The application of automated systems and robotics to mass production could potentially significantly reduce the cost. Other problems encountered while mass rearing natural enemies include the loss of genetic variability and the loss of effectiveness of species that have been raised for several generations in the laboratory (16, 25).

The performance of biopesticides in the field has often been highly variable due to environmental factors, interactions with other organisms, and poor delivery to target organism among other problems. Formulation of biopesticides (mixing of the cultured microbial preparation with inert agents to achieve proper dilution, deposition, moisture holding capacity, protection from ultraviolet rays, shelf life, slow release, etc.) must be improved to increase efficacy in the field. Long-range needs include identifying new control agents, increasing the toxicity of agents against susceptible pests, and expanding the range of hosts of the control agent (10, 16).

Delivery systems also need to be improved. Techniques must be designed to promote maximum efficacy and ease of application. New sprayer technologies, application of biopesticides by irrigation methods, and timed release formulations are needed.

Finally, a general need exists to assess the efficacy and impacts of control agents after release. Studies using biological control agents have rarely adequately documented efficacy, reliability, and economic feasibility. Population establishment and buildup, degree and timing of feeding damage, plant population density and productivity, plant stress, and nontarget side effects need to be assessed. Any changes in the fitness of the naturalized bioagent need to be ascertained to ensure efficacy and environmental safety. While these questions are pertinent to all biological control agents, they will be critical to regulatory approval of genetically engineered control agents (10, 16, 25, 38).

Use of Biotechnology in Biocontrol Research

Traditional technologies, such as chemical- or ultraviolet-generated mutations followed by selection for desired phenotypic traits, and sexual mating will continue to play a role in producing and identifying natural enemies via improved control capability or host range. Additionally, traditional culture techniques can be used to induce increased secretion of certain toxins and enzymes involved in pathogenesis. However, new biotechnology tools, such as protoplasm fusion and gene transfer, will also be used to improve virulence, sporulation, fitness for survival, infectivity under suboptimal conditions, and production of pesticidal metabolizes; and to expand host range and the tolerance of control agents to certain chemical pesticides (10, 16, 25, 38).

Biotechnology to improve biological control agents, such as insects and other arthropods, nematodes, protozoans, and fungi, is technologically more complex than biotechnology involving viral and bacterial control agents. Use of genetic engineering in predator and parasitic insects is constrained by the lack of universal vectors or other techniques to transfer foreign genes into the insect, and the lack of useful insect genes that have been cloned. Recombinant DNA techniques are being used to turn slow acting viruses into quick acting viruses, and to increase virus virulence. Genetic engineering is being used to improve the delivery of *Bacillus thuringiensis* toxin to the pest. Methods include incorporating the toxin gene into bacteria that inhabit seed coatings, roots, or surface films where target insects feed. Genetic engineering in fungi is being used to improve germination, penetration of the insect cuticle, and increase toxicity. Little biotechnology research has been conducted using protozoans and nematodes (10, 16, 25, 38). In addition to enhancing the field efficacy of biological control agents, biotech-

nology provides powerful research tools to further our basic understanding of the physiology and biology of these control agents and their environment.

Institutions Involved in Biological Control Research

Biological control research has been conducted primarily by public sector institutions, such as the U.S. Department of Agriculture (i.e., the Agricultural Research Service, the Office of International Cooperation and Development, the International Research Division, and the Forest Service), the Land Grant University System, and other public and private universities. Other Federal agencies that have supported biological control research include the U.S. Army Corps of Engineers (primarily for aquatic weeds), the Department of Interior (mainly the Park Service), the Department of Energy (through the national laboratory system), and the Tennessee Valley Authority. Selected State Natural Resources or Agricultural departments (notably those of California and Florida) also have supported biological control development. The U.S. Environmental Protection Agency is involved in registering biological control agents as pesticides. The U.S. Department of Agriculture Animal and Plant Health Inspection Service regulates the importation of natural enemies and the environmental release of biological control agents. The State Department also is involved in obtaining permission to search foreign countries for natural enemies of pests imported to the United States, and with negotiating release conditions of natural enemies with Canada and Mexico (10, 16, 25, 38).

Private industry interest has been focused primarily on organisms that can be used in microbial pesticide applications, such as *Bacillus thuringiensis* to control insects, and a few selected fungi (i. e., CASST, COLLEGO, and DeVine) to control weeds. A limited level of private-industry support exists for the use of predators and parasites to control arthropods. A few small, private firms mass rear parasites and predators for release, but conduct little or no research (10, 16, 25, 38).

Use of Biological Control Agents To Control Pests in the United States

Biological Control of Arthropods: Parasites and Predators

Arthropod (e.g., insects, spiders, mites) damage is a major contributor to crop losses and decreased quality of agricultural products. A wide array of biological control agents can be used to control arthropods, bacteria, viruses, fungi, protozoa, and nematodes. In the United States, these agents have been used to control several arthropod species (table 2-6). The classical method of control is the approach used most often, and the greatest success has occurred in more stable habitats such as forests and orchards, rather than row crops.

Table 2-6—Use of Parasite or Predator Insects To Control Insect Pests in the United States

Pest insect	Host plant
Classical method	
Rhodesgrass scale	Grasses
Citrus blackfly	Citrus
Walnut aphid	Walnuts
Cottony cushion scale	Citrus
Olive scale	Olives
Spotted alfalfa aphid	Alfalfa
Alfalfa weevil	Alfalfa
California red scale	Citrus
California purple scale	Citrus
California yellow scale	Citrus
Browntail moth	Forests
Satin moth	Forests
Oriental moth	Forests
Elm leaf beetle	Forests
European pine sawfly	Forests
European spruce sawfly	Forests
Larch casebearer	Forests
Larch sawfly	Forests
Augmentation method	
Mexican bean beetle	Soybeans
Mealybugs	California citrus
California red scale	Citrus
Spider mites	Almonds
Two spotted spider mite	Strawberries
Conservation method	
European red mite	Apples

SOURCE: Office of Technology Assessment, 1992.

Traditional selection methodologies have been used to identify parasites or predators with improved control capability or host range. For example, such techniques were used to identify strains of a parasitic mite resistant to selected pesticides, which were subsequently released into California almond orchards to control spider mites. Increased pesticide resistance allows this parasitic mite to be used in conjunction with Integrated Pest Management programs that use pesticides to control navel orangeworms above a threshold level. The ability to use this predatory mite in conjunction with other insect control programs increased the acceptance of this parasite for spider mite control (25).

Use of genetic engineering in predator and parasitic arthropods is constrained by the lack of universal vectors or other techniques to transfer foreign genes into the arthropod. Current research is focusing on the use of transposons to transfer genes, but transposons may be

Photo credit: U.S. Department of Agriculture, Agricultural Research Service.

The parasitic wasp *Microplitis croceipes* lays her eggs in the tobacco budworm. By putting this natural predator to work, scientists hope to control members of the genus *Heliothis*, which cause major damage to cotton, corn, soybeans, and other crops.

specific to certain species of insects, and thus cannot be used as a universal mechanism to transfer genes to all insect species. Another major constraint is the lack of **useful** arthropod genes that have been cloned (25).

Further development of predator and parasitic arthropods to control pest arthropods is being constrained by several factors. Selection standards for classical control approaches are needed. The economic importance of the target pest is frequently the only factor considered when selecting possible subjects for biological control. Characteristics of the natural enemy itself, such as its suitability of mass rearing at reasonable cost, additional host requirements, impact on beneficial or endangered species, or dispersal characteristics may not be considered (25).

Use of augmentation techniques to control pest arthropods with other parasitic and predator arthropods is limited by the lack of artificial diets and subsequent high cost of mass rearing, incomplete information on release methods, lack of rapid and effective monitoring methods, and lack of ability to stockpile or store natural enemies or maintain gene banks. Quality control standards for private firms that mass rear predatory or parasitic arthropods are lacking. Mixed colonies or even colonies of the wrong species have sometimes been provided; in some cases, firms have produced parasitic arthropods unable to fly. Arthropods can be sold without guidelines as to number to release, optimal timing of release, or how to monitor efficacy of release. Professional quality standards and appropriate management information are needed (25). Conservation methods to maintain predator or parasitic arthropods are constrained by gaps in the knowledge of the role of natural enemies in crop systems and how best to modify management practices to maintain natural populations.

Table 2-7—Pathogens Used To Control Insects in the United States

Pest insect	Host plant
Viruses	
European pine sawfly	Trees
Douglas fir tussock moth	Trees
Soybean looper	Soybeans
Velvetbean caterpillar moth	Soybeans
Gypsy moth	Trees
Bacteria	
Japanese beetle	Turf grass
Mosquito larvae	NA
Greater wax moth	Beehives
Fungi	
Browntail moth	Trees
Plant bug	Apples
Aphids	Potatoes
Spotted alfalfa aphid	Alfalfa
Mosquito larvae	NA
San Jose scale	Trees
Whiteflies	Trees
Protozoa	
Grasshoppers	Rangeland
European corn borer	Corn
Nematodes	
Butterflies, beetles	Cranberry, Citrus
Face fly	Cattle
Mosquito larvae	NA

NA = Not applicable,

SOURCE: Office of Technology Assessment, 1992.

Biological Control of Arthropods: Pathogens

In addition to parasitic and predatory arthropods, pathogens can be used to control pest arthropods. Pathogens that have been used to at least partially control arthropods (almost exclusively insects) in the United States include bacteria, particularly different strains in the *Bacillus genus*; viruses, particularly members of the baculovirus group; fungi; protozoans; and nematodes (table 2-7). *Bacillus thuringienses* (Bt), discussed earlier, is the pathogenic bacteria most frequently used to control insects.

The tools of biotechnology can be used to improve the delivery of the Bt toxin to insect pests. The gene that codes for the toxin can be incorporated into bacteria other than *Bacillus thuringiensis;* these bacteria may inhabit seed coatings, roots, or surface films where target insects feed. Genes coding for Bt toxins have incorporated in strains of *Pseudomonas,* a soil bacteria that colonize corn roots, and into *Clavibacter xyli,* a plant-associated (endophytic) bacterium that grows in the vascular tissues of

Photo credit: U.S. Department of Agriculture, Agricultural Research Service.

Entomologist compares an insect ravaged cotton leaf from a control variety with one that has been genetically engineered with a protective gene from *Bacillus thuringiensus.*

plants. The Monsanto and Mycogen Corp. are incorporating Bt toxin genes into *Pseudomonas,* while Crop Genetic International is working with *Clavibacter (1,* 16).

Genetic engineering techniques are also being used to modify Bt toxin genes to be toxic to a broader range of pests and to be more potent. Traditional selection and screening procedures applied to natural isolates are being used as well, to identify strains of *Bacillus* bacteria that are either more efficacious or that have different host specificity. These methods will potentially extend Bt use to include control of cotton bollworm, European corn borer, and corn rootworms. Genetically engineered and new, naturally selected strains of Bt are expected to be commercially available by 1995 (1, 16).

Viruses are also being used to control insects. Many types of viruses infect insects, but only a few cause pathogenic epizootic diseases that are sufficiently fast-acting and widespread to be considered useful for pest control. The first virus to be registered by EPA and produced commercially as a pesticide was a type of baculovirus that forms large polyhedral occlusions within the nucleus of infected cells. It was marketed in the mid 1970s by the Sandoz Corp. under the name Elcar, and was used to control cotton bollworm. Its market was displaced by the new pyrethroid pesticides. It has not been remarketed, although increasing resistance to pyrethroids may lead to renewed commercial interest. Three other baculoviruses have been used by the U.S. Forest Service to control the Douglas fir tussock moth, the gypsy moth, and the European pine sawfly (16).

Baculoviruses are used to control lepidopterans (butterflies and moths) because they cause widespread lethal epizootic diseases, lead to morbidity within a week of infection, are compatible with other agrichemicals, can be applied by conventional spraying techniques, and are stable on the shelf for extended periods of time (years). Further, the baculoviruses replicate only in arthropods. Each is specific to a host or group of closely related hosts, and must enter and replicate within a specific type of host cell. This specificity is attractive from an environmental control perspective (1).

Two other viruses of potential usefulness for biological control of insects are the *Autograph californica virus* and the codling moth granulosis virus. *A. californica* has a relatively wide range of hosts and could be used to control alfalfa looper, cabbage looper, fally armyworm, beet armyworm, and wax moth. The codling moth granulosis virus could be used to control insects that affect pome fruits and walnuts (16).

Genetic engineering is being used to make viral pesticides faster acting. Neurotoxin genes that paralyze the pest insect and quickly halt insect feeding are being introduced into baculovirus. Alternatively, insecticidal hormones can be incorporated into the baculovirus to disturb insect development or behavior. The genes that code for an enzyme that regulates juvenile hormone levels in insects; a protein that regulates the release of a major molting hormone; and a protein hormone that elicits several behavioral characteristics during molting all recently have been isolated (1).

The lack of suitable cloned neurotoxins and insect hormone genes is delaying further progress in improving viral control agents. Promotors that can be recognized by selected host cells of pest insects (i.e., cells of the midgut, for example) are being used to extend baculovirus ranges. The recent discovery that baculoviruses normally contain a gene regulating insect molting hormone activity is leading to the development of baculovirus strains in which this gene has been deleted. These gene-deleted strains have been shown to reduce insect feeding during infection, and to hasten the onset of insect morbidity (1).

Baculoviruses genetically modified to delete the insect molting hormone regulatory gene are expected to be available before 1995. Baculoviruses engineered to carry

Table 2-8—Control of Weeds by Insect and Microbial Agents in the United States

Weed	Habitat/crop affected
Alligator weed	Aquatic
Lantana	Rangeland, forest, crops
Musk thistle	Rangeland
Northern jointvetch	Rice and soybeans
Persimmon	Rangeland
Prickly pear cactus	Rangeland
Puncture vine	Pasture, annual crops
Skeletonweed	Rangeland
St. Johnswort	Range and arable lands
Stranglervine	Citrus
Water hyacinth	Aquatic

SOURCE: Office of Technology Assessment, 1992.

insecticidal genes such as insect hormones and neurotoxins could be available in the late 1990s.

The only fungus registered and commercially produced for insect control in the United States was *Hirsutella thompsonii.* This fungus was used to control citrus rust mites, **but** was not commercially successful primarily **because it did** not survive storage or transportation. Further, environmental factors, including insufficient moisture, adversely affected its efficacy. Genetic engineering of fungi is now being used to improve germination, improve penetration of the insect cuticle, and increase toxicity (16).

A major limitation to using protozoans is that they kill insects very slowly, if at all. Generally they affect arthropods by causing chronic disease with sublethal effects, reducing the ability of the arthropod to survive the winter. *Nosema locustae,* used to control grasshoppers on rangeland, is the only protozoan to be registered and commercially available in the United States (16).

Research involving nematodes has been increasing. *Steinernema carpocapsae* has been used in the United States to control some lepidoptera species. It is not effective if applied to vegetation surfaces or other situations where it can dry out, but it can be effective in the soil or in burrows in plant tissues. *Dedalenus siricidicola* has been used to control woodwasps, even though its action is to sterilize its host rather than kill it. Very little genetic engineering is being used with nematodes.

Biological Control of Weeds: Microorganisms and Arthropods

Historically, biological control of weeds most commonly has been mediated by microorganisms (mainly bacteria and fungi, see table 2-8) and insects. Worldwide, 89 species of weeds have been controlled using 192 species of introduced organisms (the classical approach); an additional 25 weed species have been controlled using 33 species of native organisms (the bioherbicide approach) (10).

Pathogenic microorganisms kill or severely debilitate their host plants by causing disease. Pathogenic and nonpathogenic microbes also produce metabolizes that are toxic to plants, and these phytotoxins can also be used as herbicides. For example, the fungus *Gliocladium virens,* when prepared and applied properly, can release enough of the toxin viridiol in the soil to control pigweed without harming cotton seedlings.

The private sector has shown interest in developing microbial herbicides. Two microbial herbicides (COLLEGO and DeVine) are commercially available and four others are undergoing trials for registration as herbicides (table 2-9). Other microbial herbicide candidates are undergoing experimental development. About 107 fungi and 1 bacterium are being evaluated worldwide as bioherbicides (10). Additionally, a parasitic nematode, *Orrina phyllobia,* has been shown to be a practical means to control silverleaf nightshade.

Development of a microbial herbicide can take several years. For example, it is estimated that the development of COLLEGO® took 11 years of effort from the time of discovery to commercial availability at a cost of about $1 to $1.5 million. In comparison, a typical chemical herbicide takes 7 to 10 years to develop and costs approximately $80 million. Early research on microbial herbicides is subsidized by public funds, but the expense of large-scale fermentation, toxicology testing, formulation, and registration are borne by industry. In some cases, these costs could prove to be quite high (10). Further development of microbial herbicides will require improved mass production, formulation, and delivery systems. Some native pathogens, such as the rusts and certain smut fungi, cannot be artificially grown. Methods to obtain sufficient quantities of these pathogens from infected plants must be developed.

Weed pathogens are being genetically manipulated to improve virulence, sporulation, fitness for survival and infection under suboptimal conditions, and production of herbicidal metabolizes; to expand host-range; and to increase tolerance to certain chemical pesticides. For example, it has been discovered that altering a single enzyme *(pisatin demethylase)* **can** cause a fungal pathogen, but not a nonpathogenic fungi, to become virulent on new host plants. Genetic engineering techniques are also being used to increase virulence by transferring genes encoding herbicidal phytotoxins to pathogenic microorganisms (10).

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Table 2-9—Microbial Herbicides Commercially Available or in Development in the United States

Herbicide	Pest	Crop/habitat effected
COLLEGO®	Northern jointvetch	Rice
DeVine®	Stranglervine	Citrus
CASST™[a]	Sicklepod	Soybean and peanut
BioMal™[a]	Round-leaf mallow	Annual crops
Cercospora rodmanii[a]	Waterhyacinth	Aquatic
Mycoleptodiscus terrestris[a]	Eurasian watermilfoil	Aquatic

[a]Undergoing trials

SOURCE: Office of Technology Assessment, 1992.

Table 2-10—Use of Insects To Control Weeds in the United States

Weed	Crop/habitat affected
Classical approach	
St. Johnswort	Range and arable lands
Lantana	Rangelands, forests, and plantation crops
Alligatorweed	Aquatic
Prickly pear cactus	Rangeland
Puncturevine	Pastures and annual crops
Tansy ragwort	Rangeland
Hydrilla	Aquatic
Purple loosestrife	Range and arable lands
Leafy spurge	Rangeland
Diffuse, spotted and Russian knapweeds	Rangeland
Yellow starthistle	Rangeland
Salt cedar	Rangeland and forests
Field bindweed	Various crops
Waterlettuce	Aquatic
Broom snakeweed	Rangeland
Baccharis neglecta	Range and arable lands
Augmentation approach	
Waterlettuce	Tried and discontinued
Purple nutsedqe	Tried and discontinued

SOURCE: Office of Technology Assessment, 1992.

Traditional techniques are also used to alter pathogen characteristics. These include chemical- or ultraviolet-generated mutations followed by selection for desired phenotypic traits, breeding, and nonsexual transfer of hereditary properties. Cultural techniques also are being improved to increase secretion of certain toxins and enzymes involved in pathogenesis.

In addition to microbial pathogens, insects and other arthropods also can be used as biological control agents for weed control (table 2-10). The relationship between insects and weeds is complex. Some weeds (e. g., St. Johnswort) can be controlled with just one insect. Others may require more than one insect for control. For example, control of tansy ragwort, a poisonous weed found in the Pacific Northwest, is mediated by a moth that defoliates it and a second insect that feeds on its root as a larva and on the resprouting growth as an adult. This relationship between each co-evolved arthropod and its weed host makes each study unique and raises the question of whether scientific expertise will ever be adequate to fully assess the potential for weed control by arthropods (10).

Arthropod adults and immature larva and nymphs feed and complete at least a part of their life cycles on certain weeds. In this process, they damage the plants, weakening and reducing their productivity and competitiveness. In general, the feeding activity of immature arthropods is more damaging than that of adult arthropods. The extent of the damage caused by arthropod feeding depends on the particular weed tissues destroyed, the timing of the damage as it relates to the plant's growth cycle, and the extent of other plant stresses present. For example, sucking insects and grasshoppers defoliate plants late in the plant's life cycle and do not cause as much damage as insects that defoliate plants early in their life cycles. Arthropods that attack the seeds of weeds that cannot reproduce vegetatively are likely to have the greatest impact on weed control. In addition to feeding damage, some arthropods weaken plants by introducing toxins causing cell proliferation and gall formation (10).

Of the more than 250 naturalized plant species considered to be major weeds, only a few dozen have been considered for classical biocontrol by arthropods. Nonetheless, this approach has been the most common and successfully used method of biological weed control. It is estimated that the control of St. Johnswort by insects has yielded benefits worth approximately $2 million per year. It takes 1 to 4 years to find and clear each insect or other arthropod biocontrol candidate and development costs are estimated at $1 to 2 million. However, the estimated return on research is about $30 for every $1 invested (10). Few attempts to control weeds with ar-

Photo credit: U.S. Department of Agriculture, Agricltural Research Service.

Tiny (1/8th inch long) flea beetle, *Apthona flava,* on leafy spurge is one of several biological control agents tested to combat a costly weed that infests 2½ million acres of rangeland in the Great Plains.

thropods using the augmentation approach have been tried, and generally they have been discontinued.

Traditional selection methods are used to select cold-tolerant strains of weed-damaging insects and strains whose larva have higher survival rates in hot weather, and whose prediapause behavior has been altered. Genetic engineering is not currently used to improve arthropods as biological control agents (10).

Biological Control of Disease

Biological control of plant diseases is achieved by decreasing pathogen populations or by preventing the occurrence of infections. Approaches taken include manipulating resident microbial communities to decrease disease (conservation approach) or applying to the plant organisms antagonistic to pathogens (augmentation). Only three plant disease biocontrol agents are commercially available (table 2-11) (38).

Table 2-1 l—Biological Control Agents Commercially Available To Control Plant Disease in the United States

Agent	Disease controlled
Bacteria	
Agrobacteriurn radiobacter (strain K84)	Crown gall in dicots
Pseudomonas fluorescent.	Damping off and root rot in cotton
Fungi	
Peniophora gigantea.	Root and butt rot in conifers

SOURCE: Office of Technology Assessment, 1992,

Use of *Agrobacterium radiobacter* to control crown gall in dicots costs an estimated 1 to 5 cents per plant treated, and less if the seeds are treated. *Peniophora giganted* applied to freshly cut conifer stumps preempts colonization by the pathogen responsible for root and butt rot, diseases resulting in annual losses of nearly $1 billion. *Pseudomonas j7uore.seen.s,* sold under the name of Dagger G by Ecogen, controls diseases in cotton. In 1989, it was used on approximately 75,000 acres of cotton in the Mississippi Delta region (38).

Diseases that potentially could be controlled in the next decade include take-all disease in wheat, and damping-off and root rot in crops other than cotton. Yeasts to suppress *Penicilliurm* and other postharvest pathogens in citrus and other fruit; the bacterium *Bacillus subtilis* to control brown rot in peaches; and compost amended potting media to control *Rhizoctonia* and *Pythium* in nursery stocks are other potential control agents (38).

The use of microbial disease control agents has been plagued by inconsistent efficacy in the field. In some cases, agents that have worked in one field have failed to be effective in immediately adjacent fields. The biocontrol agent and pathogen interact in the midst of a vast array of other microorganisms that sometimes decrease the efficacy of the control agent *(23, 24).* A better understanding of the community dynamics, population and community ecology, population genetics of plant-associated microorganisms and of the mechanisms that regulate the community structure and dynamics of plant-associated microorganisms is needed.

Much of the research in the area of biocontrol of plant diseases has focused on improving the understanding of the mechanisms by which biocontrol agents prevent disease. One mechanism of action called interference competition or antibiosis refers to the inhibition of one organism by a metabolic product of another. The use of the bac-

terium *Agrobacterium radiobacter* strain K84 to control crown gall tumors caused by *Agrobacteriurm tumefaciens* is an example of this type of mechanism. *A. radiobacter* produces an antibiotic to *A. tumefaciens.* Control of take-all disease in wheat by ***Pseudomonas fluorescens*** strain 2-79 is another example of antibiosis (38).

Peniophora gigantea controls root rot in pine caused by the fungus *Heterobasidion annosum,* on the other hand, by competing with the fungus for nutrients and space, a process referred to as exploitation competition. A third mechanism, hyperparasitism, occurs when fungal pathogens destructively parasitize another organism. **Fungi of the** *Trichoderma* and *Gliocladium* family, for example, parasitize soil-born plant pathogens such as *Rhizoctonia solani and Pythium* species. A fourth mechanism of disease prevention by biological agents is hypovirulence. For example, some strains of the chestnut blight fungal pathogen *Cryphonectria parasitic* (those with reduced virulence) can impart protection to chestnut trees from more virulent strains of this pathogen.

Traditional screening techniques are being used to develop fungicide-resistant strains of the fungus *Trichoderma,* which allows this disease control agent to be used with fungicides so that fewer chemicals need be applied. Strains of the bacteria *Pseudomonas syringae* pv. tomato, which controls bacterial speck in tomatoes, have been made resistant to copper. The copper resistance allows *P. syringae* to be used in the presence of copper bactericide. Combinations of *P. syringae* and copper bactericide gives greater control over bacterial disease than occurs with the biocontrol agent or bactericide treatment alone (31, 32).

Pathogenic organisms can become resistant to biological control agents. For example, *A.* ***radiobacter*** controls the plant pathogen *A. tumefaciens* **by** producing a compound called agrocin. The gene producing agrocin is carried on a plasmid, which can be naturally transferred to *A. tumefaciens.* Thus, *A. fumefaciens is* becoming resistant to *A. radiobacter.* Genetic engineering is being used to construct mutant strains of *A. radiobacter* that no longer have the ability to transfer the agrocin plasmid, thus decreasing the potential of *A. tumefaciens* to develop resistance to this natural pesticide. Protoplasm fusion techniques are also being used to construct strains of *Trichoderma harzianum* that are more effective than parental strains in controlling *Pythium ultimum (38).*

Biological Control of Frost Damage

The temperature at which frost injury occurs in a number of crops is determined by the population density of ice-nucleation-active bacteria on plant leaves. By decreasing the numbers of these bacteria, some protection against frost damage can be achieved. The application of non-ice-nucleating bacteria prior to colonization of ice-nucleating bacteria can effectively prevent the establishment of the ice-nucleating bacteria by limiting the resources (i.e., space and/or nutrients) available to the ice-nucleating bacteria. Ice-minus deletion mutants of the bacteria *Pseudomonas syringae* have been constructed to control frost. The first planned introductions of genetically engineered bacteria into the environment in the United States involved the field-testing of these ice-minus bacteria.

SUMMARY

Pest control is a major concern of crop producers in the United States. Each year, pest damage results in billions of dollars of lost revenue to farmers. Poor weather conditions add to those losses. To control pest damage, farmers have traditionally used chemical approaches. Biotechnology is now providing opportunities to use biological approaches such as transgenic plants resistant to pests and better adapted to geoclimatic conditions, and the use of biological control agents.

The ability to create transgenic plants with useful agronomic characteristics is constrained by the lack of knowledge concerning plant physiology. Our understanding of plant metabolism has not kept up with the development of biotechnology methods. However, plants resistant to certain insects are approaching commercialization. Most of these plants have a *Bacillus thuringiensis* toxin gene insert, **but** some research also is being conducted using insect trypsin inhibitors that disrupt the digestion of feeding insects. Several transgenic Bt plants are undergoing field trials, and it is expected that several companies will begin petitioning EPA for approval for commercial release soon.

Plants tolerant of herbicides are being developed to aid the management of weeds. Development of broad-spectrum herbicides has been constrained because they not only kill most weeds, but also cause significant damage to crops. Crops tolerant to specific herbicides allow the use of these herbicides in conditions where they previously could not be used, and may allow for the replacement of some environmentally damaging herbicides. Some of these crops are nearing commercialization stages.

Transgenic plants are being developed that are resistant to disease. Scientific understanding of the complex interactions between fungi or bacteria and host plants is limited, so much of the early successes have been in

developing plants resistant to certain viral diseases. Several virus-resistant plants are under development.

Development of transgenic plants tolerant to geoclimatic conditions is in the early stages. Research is being conducted to understand the mechanisms of heat and drought tolerance, and to enhance the ability of plants to withstand cold temperatures. However, the successful commercialization of these plants is unlikely to occur before the end of the decade.

In addition to engineering crops themselves, there is increased interest in developing biological control agents to manage pests. The use of biological control in the United States, to date, is relatively limited and most successes have involved controlling pests in forests, orchards, grasslands and aquatic environments. Use of biological control in grain and row crops is very limited. However, there is more emphasis placed on developing such products to control weeds, insects, and disease in the major food crops, and improved strains of *Bacillus thuringiensis* to control insects and a few fungal strains to control weeds are approaching commercialization. More research still is needed to successfully develop other products.

The food processing industry will also be affected by biotechnology. Plants are modified for new quality and processing characteristics. For instance, tomatoes with delayed softening characteristics are nearing commercialization. Research is also underway to alter the starch, oil, and protein content of selected crops to more closely reflect consumer preferences and to enhance their processing characteristics for specific end uses. Diagnostic kits are in various stages of development to detect the presence of microorganisms, chemicals, and other contaminants in food products.

The development of transgenic plants and biological control organisms offer new approaches to controlling pests and to improving food processing characteristics. However, many issues have been raised concering the development of these products. Some groups are worried about the effects these products will have on small farms, and on food safety and the environment. Additionally, many of these products will require extensive farm management capabilities for effective use. These issues will be discussed in subsequent chapters.

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