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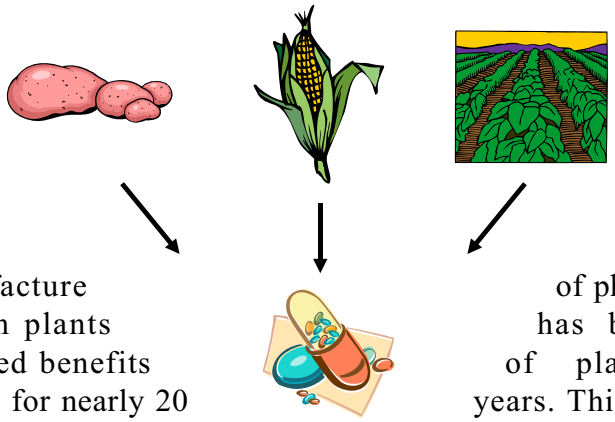
FROM THE GROUND UP

Agronomy News

Bio-Pharming



INSIDE THIS ISSUE

The manufacture of pharmaceutical products in plants has been among the promised benefits of plant genetic engineering for nearly 20 years. This application of biotechnology, sometimes known as "pharming", "bio-pharming", or "molecular farming," has now moved beyond the realm of speculation into the experimental testing phase in fields and greenhouses. Bio-pharming promises more plentiful and cheaper supplies of pharmaceutical drugs, including vaccines for infectious diseases and therapeutic proteins for treatment of conditions such as cancer and heart disease. "Plant-made pharmaceuticals" (PMPs) are produced by genetically engineering plants to produce specific compounds, generally proteins, which are extracted and purified after harvest. (For an introduction to plant genetic engineering, please visit the web site "Transgenic Crops: An Introduction and Resource Guide," http://www.colostate.edu/programs/lifesciences/TransgenicCrops/)

A variation of PMP technology is to infect plants with viruses that are engineered with the gene for the pharmaceutical protein. Upon infection, the plant's cellular machinery produces the biopharmaceutical along with other viral proteins (Freese, 2002). As used here, the terms bio-pharming and PMP do not include naturally occurring plant products or nutritionally enhanced foods.

Although PMP technology offers potential health and economic benefits, all observers agree that it must be strictly regulated to prevent pharmaceuticals from entering the food supply and to avoid unintended effects on the environment. The following information, presented in question and answer format, covers basic information on the production, regulation, risks, and benefits of PMPs.

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How are drugs currently manufactured?

Many protein-based drugs are currently produced in sterile fermentation facilities, where micro-organisms or mammalian cell cultures in stainless steel tanks churn out a range of genetically engineered products (Felsot, 2002). Because these facilities have huge capital construction costs, industry has been unable to keep up with the growing demand. Other drugs are extracted from animal organs, a high-cost procedure that carries the risk of transmitting infectious diseases to humans. Due to advances in plant genetic engineering over the past two decades, plants can now be modified to produce a wide range of therapeutic products at a price significantly cheaper than through current methods. For example, antibodies that currently cost thousands of dollars per gram might be produced in plants for $200 per gram (Ohrlogge and Chrispeels, 2003).

What pharmaceuticals could be made in plants?

At least for the near-term, PMPs will be proteins. Because proteins are directly encoded by genes, their production through genetic engineering is more straightforward than other types of biochemical compounds, which are synthesized via more complex biochemical pathways. Some potential bio-pharm products are listed in Table 1.

What crops are being considered for pharmaceutical production?

The most commonly mentioned host plants or "Pharm Crops" for PMP production are corn, tobacco, and potato. Other crops being investigated include alfalfa, rice, safflower, soybean, and tomato. Suitable host plants must be easily engineered, be capable of high levels of protein production, and have appropriate procedures for extracting the PMP from plant tissues. Knowledge of the agronomy, physiology, pests and diseases of a crop is also an advantage. Ideally, the host plant would be a non-food crop such as tobacco that does not have wild relatives present in the production environment. Another desirable feature is a biological mechanism (such as self-pollination or male sterility) that minimizes pollen drift to nearby fields of the same crop.

What part of the plant will produce the PMP?

Most bio-pharming applications target production and storage of the engineered product in seeds, which naturally accumulate high concentrations of proteins and oils. Seeds are also the easiest part of the plant to store and transport to processing facilities. Seed-specific promoters used in experimental bio-pharm lines include the beta-phaseolin promoter of common bean and the oleosin promoter of *Brassica* species (Moloney, 2000). (Promoters are regulatory elements of genes that control how much of a gene product is made and where in the plant it is synthesized.) The location of protein accumulation within the cell is also important in ensuring correct folding and stability of the protein (Moloney, 2000). Not all PMPs will be produced in seeds; leaves are the target tissues in some alfalfa and tobacco applications, and tubers are targeted in potato production systems (Canadian Food Inspection Service, 2001).

How will PMPs be produced?

Pharmaceutical production in plants will be a highly sophisticated and closely regulated enterprise, and will be very different from conventional crop production in many ways. Bio-pharm crops must be grown, transported, and processed using safeguards designed to prevent

Continued on 4

FROM THE GROUND UP

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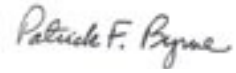
Web Site: http://www.colostate.edu/Depts/SoilCrop/extension/Newsletters/news.html



Pat Byrne
Technical Editor

Direct questions and comments to:
Deborah Fields
Phone: 970- 491-6201
Fax: 970-491-2758
E-mail: dfields@lamar.colostate.edu

Table 1. Potential plant-made pharmaceuticals.

Product	Definition	Examples
Antibodies	Specialized proteins of the immune system that initiate the body's defense response.	Specific antibodies could be developed to fight cancer, HIV-AIDS, hepatitis, malaria, dental caries, and other diseases.
Antigens (vaccines)	Compounds that elicit the production of antibodies that protect against disease.	Plant-made vaccines are currently under development for protection against cholera, diarrhea (Norwalk virus), and hepatitis B.
Enzymes	Proteins that catalyze biochemical reactions.	Enzymes could be used both to treat and to diagnose disease. For example, lipase is an enzyme that breaks down dietary fats and is used to treat cystic fibrosis and other diseases.
Hormones	Chemical messengers active at low concentrations and produced in specialized cells.	Insulin is produced in the pancreas and helps regulate sugar metabolism. Diabetics with insulin deficiencies must replace it via shots or pumps.
Structural proteins	Proteins that provide structural support to cells or tissues.	Collagen is a structural protein found in animal connective tissues and used in cosmetics.
Anti-disease agents	A wide variety of proteins.	The anti-infection agents interferon and lactoferrin and the blood anti-coagulant protein hirudin have been engineered in plants.

inadvertent mixing with food or feed crops. Some of the features that will distinguish bio-pharming from bulk commodity production are listed below and shown in Fig. 1 (Felsot, 2002; APHIS, 2003):

- All workers must receive training in the principles and methods of gene containment.
- Equipment for planting and harvesting of bio-pharm crops must be dedicated to that purpose, i.e., the equipment cannot be used with any other crop. Tractors and tillage equipment must be thoroughly cleaned before being used with other crops.
- Production fields will be carefully chosen to provide the required isolation distances from other fields of the same crop. For example, bio-pharm corn must be isolated by at least one mile from other corn fields if it is open-pollinated, and by one-half mile if pollination is controlled through male sterility or detasseling. The one mile distance is eight times the required isolation distance for certified seed corn production.
- Seed will only be available to contract growers.
- Containers used for transportation of seed to the field and harvested products to the processing plant must be labeled, sealed, and thoroughly cleaned after use.
- Bio-pharmed fields will be closely monitored during the growing season and in following seasons to ensure that required procedures are being followed and that volunteer plants are found and disposed of properly.

When will plant-made pharmaceuticals reach the market?
After many years of research in laboratories and greenhouses, a few bio-pharm crops are now being grown in experimental field plots. Plant-produced antibodies are currently undergoing evaluation in clinical trials and may reach the market as early as 2005 (Ohrlogge and Chrispeels, 2003), assuming their efficacy and safety are demonstrated, and environmental concerns are adequately addressed.

Who is doing bio-pharming?
Several multinational biotechnology firms that produce other types of genetically engineered crops (including Dow Agroscience, Monsanto, and Syngenta) are also pursuing commercial development of PMPs. A number of smaller companies (including CropTech, Large Scale Biology Corporation, Meristem Therapeutics, and Prodigene Inc.) are also leaders in the biopharmaceutical industry. These companies will most likely contract with a limited number of highly skilled farmers to produce PMP crops.

What are the benefits of plant-made pharmaceuticals?

- As mentioned previously, PMPs can be produced at a significantly reduced cost compared to current production methods. Therefore, the technology has the potential to benefit medical patients in all countries, and may be especially important for developing countries by providing a more affordable source of vaccines and pharmaceuticals. However, it is not clear how large the cost reduction will be or how much of the savings will be passed on to consumers.
- Plants can be engineered to produce proteins of greater complexity than is possible with micro-organisms (Collins, 2003), and to produce proteins that cannot be produced in mammalian cell cultures (Anonymous, 2002).
- A limited number of growers and communities will likely benefit economically from this new agricultural enterprise. The number of acres required to produce a year's worth of a given pharmaceutical will likely be quite small compared to crop acreage for food and feed use.

What are the risks of plant-made pharmaceuticals?
Risks will not be uniform for all bio-pharm applications, but will vary depending on the nature of the pharmaceutical product, the crop and tissues in which the PMP is produced, and the environment in which the crop is grown. The major risk factors of PMPs are summarized below. For a more detailed discussion, see documents by the Canadian Food Inspection Service (2001) and Freese (2002).

- Pollen from plants engineered to produce pharmaceuticals may fertilize nearby food or feed crops of the same species. If this occurs, the pharmaceutical may be produced in seed of the neighboring crop, with potentially negative effects on human or animal consumers of the seed. The risk of gene flow via pollen drift is greater in cross-pollinated crops like corn. Methods to minimize this risk include spatial and temporal

Continued on 5

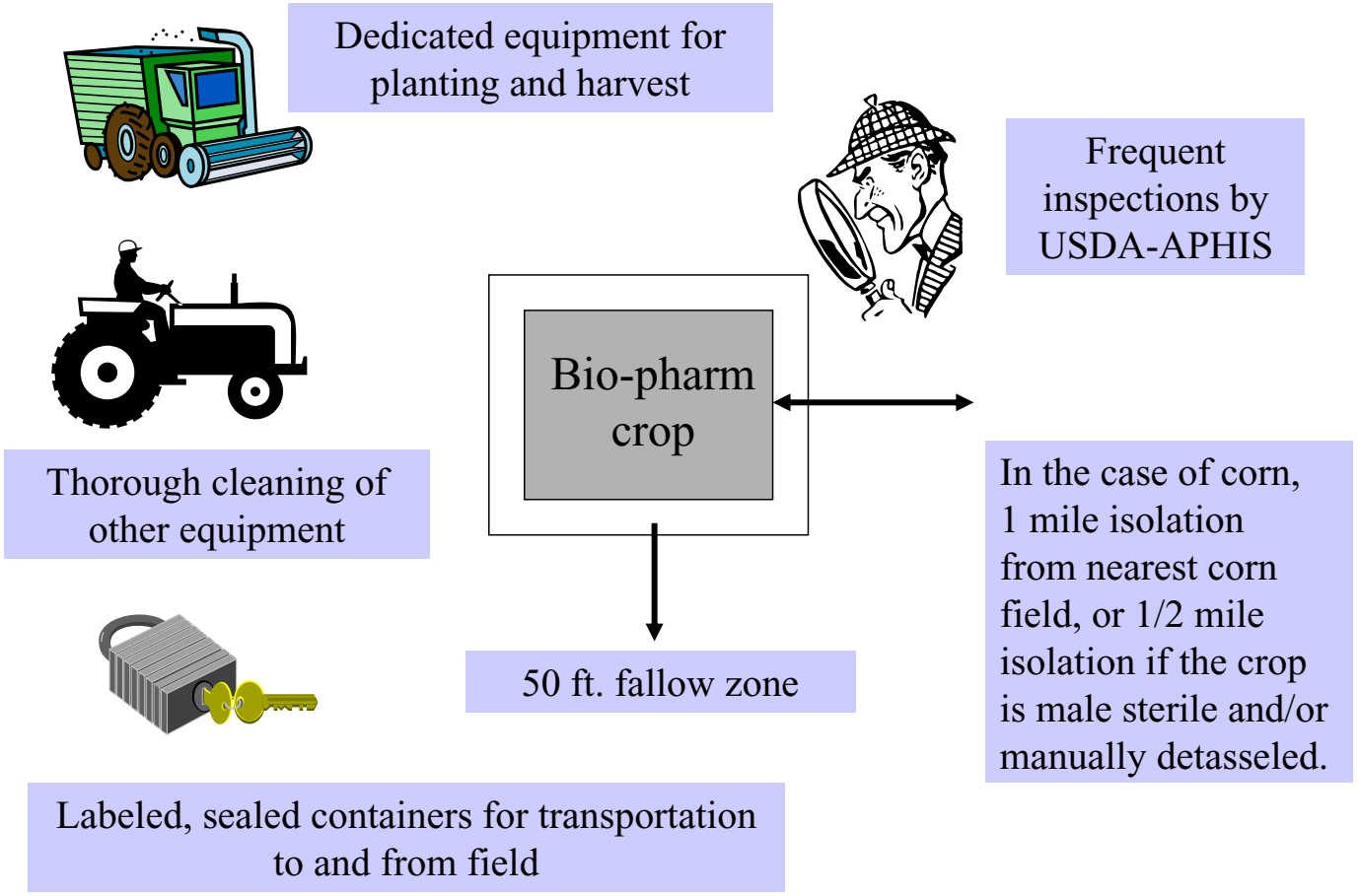


Figure 1. Some of the safeguards required by USDA-APHIS for the production of bio-pharm crops.

isolation, the use of male sterility (i.e., plants that don't produce viable pollen), and in the case of corn, detasseling (removing tassels before they shed pollen). When male sterility or detasseling are used, fertile male plants that do not produce the pharmaceutical are planted in the field to provide the pollen source.

- Co-mingling of PMP crops and food or feed crops may occur. This could happen through improper labeling, mixing of seed in planting, harvesting, transportation, or processing equipment, or the presence of "volunteer" PMP plants in subsequent seasons in the same field. In a recent case, USDA fined Prodigene $250,000 for failure to eliminate volunteer bio-pharm corn plants from a soybean crop planted later in the same field as the PMP corn (Anonymous, 2003). The company was also required to reimburse the government $3 million for expenses related to destruction of 500,000 bushels of contaminated soybeans.
- The introduced gene or its product may have negative effects on the natural environment. For example, wildlife feeding on the crop may ingest harmful levels of the PMP, or soil micro-organisms may be inhibited by decomposing crop residue or substances exuded from roots of PMP plants.
- Farm workers may be exposed to unhealthy levels of a biopharmaceutical by absorbing products from leaves through their skin or by inhaling dust at harvest.

How are pharmaceutical crops regulated?

Because bio-pharm crops are genetically engineered, they are subject to the U.S. federal regulations that govern all such crops. Three federal agencies, the U.S. Department of Agriculture - Animal and Plant Health Inspection Service (APHIS), the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA), all play

Continued on 6

roles in regulating genetically engineered crops, though their specific responsibilities vary depending on the type of application involved. (For a detailed description of the roles of the three federal agencies, see the "Evaluation & Regulation" section of the Transgenic Crops web site (http://www.colostate.edu/programs/lifesciences/TransgenicCrops/).

Besides the standard regulations, bio-pharm crops are subject to additional regulatory oversight. In March, 2003 APHIS announced more stringent conditions for field tests of genetically engineered crops that produce pharmaceutical or industrial compounds. Several of these new requirements are listed in the previous section entitled "How will PMPs be produced?" and in Fig. 1. The objective of these regulations is to prevent any contamination of food and feed crops with the bio-pharmaceuticals and to minimize environmental impacts. In recognition of the evolving status of federal regulation of PMP crops, APHIS has invited public comment on ways to make the regulatory process more transparent, improve field test confinement, and enhance monitoring and compliance. A discussion of the adequacy of APHIS' new regulations is available on the Pew Initiative on Food and Biotechnology web site (Anonymous, 2003).

FDA has the responsibility to ensure the safety and efficacy of drugs. Therefore, clinical trials and marketing of PMPs will require FDA approval. FDA will also oversee procedures for manufacturing PMPs to guarantee consistent product quality and potency.

EPA will become involved in the regulatory process if the PMP crop contains engineered insect resistance, such as Bt insecticidal proteins. If questions arise about the environmental impact of bio-pharming that are not addressed by the other agencies, then EPA has options for intervening on that issue.

The department of agriculture of the state in which a PMP crop field test is proposed, is given the opportunity to review APHIS' preliminary assessment of applications for field testing of genetically engineered crops. In the past, this has been a routine approval, but with PMP crops, states are taking a much more cautious approach. State departments of agriculture may well request additional permit conditions beyond those imposed by APHIS.

Are bio-pharm crops likely to be grown in Colorado in the near future?

Among the advantages Colorado has for bio-pharming are the possibility of achieving greater isolation distances for corn, compared to many midwestern locations, and the ability to obtain high yields under irrigated conditions with relatively little disease and insect pest pressure. Apparently recognizing these advantages, one company has applied to APHIS for a permit to grow a field test of PMP corn in Colorado in 2003. According to Mitch Yergert of the Colorado Department of Agriculture (CDA), APHIS has reviewed and approved the application and forwarded it to his department for review. To assist with the evaluation of this and future permit applications for PMP crops, the CDA has formed a Technical Advisory Committee, which will evaluate the adequacy of conditions for gene containment and for minimizing environmental impact. At press time, no decision had yet been made by the CDA on the 2003 application.

Final thoughts

Before bio-pharm crops become a successful commercial venture, several major hurdles must be overcome. First, the safety and efficacy of drugs produced in plants need to be demonstrated. Second, the appropriate genes, crop species, plant parts, and confinement conditions for growing these crops, both from technical and regulatory points of view, must be determined. After the StarLink experience (http://www.colostate.edu/programs/lifesciences/TransgenicCrops/hotstarlink.html) and the recent ProdiGene episode, regulatory agencies will be extremely wary of the risks of cross-pollination or co-mingling of PMP crops with food or feed crops, so confinement conditions will be strict. Third, production costs for PMPs, especially the costs of purification, must be reduced before bio-pharm crops become economically feasible. Finally, consumers must be willing to accept this new source of pharmaceutical products. When, or if, some bio-pharm crops are approved, they will likely provide new business opportunities for a small number of growers, rather than an economic bonanza for rural areas.

by Pat Byrne
Extension Agronomy Specialist
Assistant Professor
Colorado State University

Continued on 7

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Additional sites for information on PMP crops:

The Union of Concerned Scientists web site has an interactive feature that discusses benefits and risks of pharm crops http://www.ucsusa.org/pharm/pharm_open.html. The site includes a list of companies (with web links) that are involved in PMP technology.

Colorado State University Cooperative Extension

Colorado Wheat Field Days 2003

Stratton	*June 3 (Tues)*	*5 p.m. at Miltenberger Bros. Farm, Kit Carson County*
Walsh	*June 9 (Mon)*	*9 a.m. at Plainsman Research Center, Baca County*
Lamar	*June 9 (Mon)*	*5 p.m. at John Stulp's house, Prowers County*
Sheridan Lake	*June 10 (Tues)*	*9 a.m. at Eugene Splitter Farm, Kiowa County*
Cheyenne Wells	*June 10 (Tues)*	*1 p.m. at Tom Heinz Farm, Cheyenne County*
Burlington	*June 10 (Tues)*	*5 p.m. at Barry Hinkhouse Farm, Kit Carson County*
Genoa	*June 11 (Wed)*	*9 a.m. at Ross Hansen Farm, Lincoln County*
Julesburg	*June 11 (Wed)*	*3 p.m. at Walt Strasser Farm, Sedgwick County*
Ovid (Irr)	*June 11 (Wed)*	*5 p.m. at Jim Carlson Farm, Sedgwick County*
Orchard	*June 12 (Thurs)*	*9 a.m. at Cary Wickstrom Farm, NW Morgan County*
Briggsdale	*June 12 (Thurs)*	*11:30 a.m. at Stan Cass Farm, N Weld County*
Bennett	*June 17 (Tues)*	*5 p.m. at John Sauter Farm, Adams County*
Akron	*June 18 (Wed)*	*8 a.m. at Central Great Plains Res. Station, Washington County*