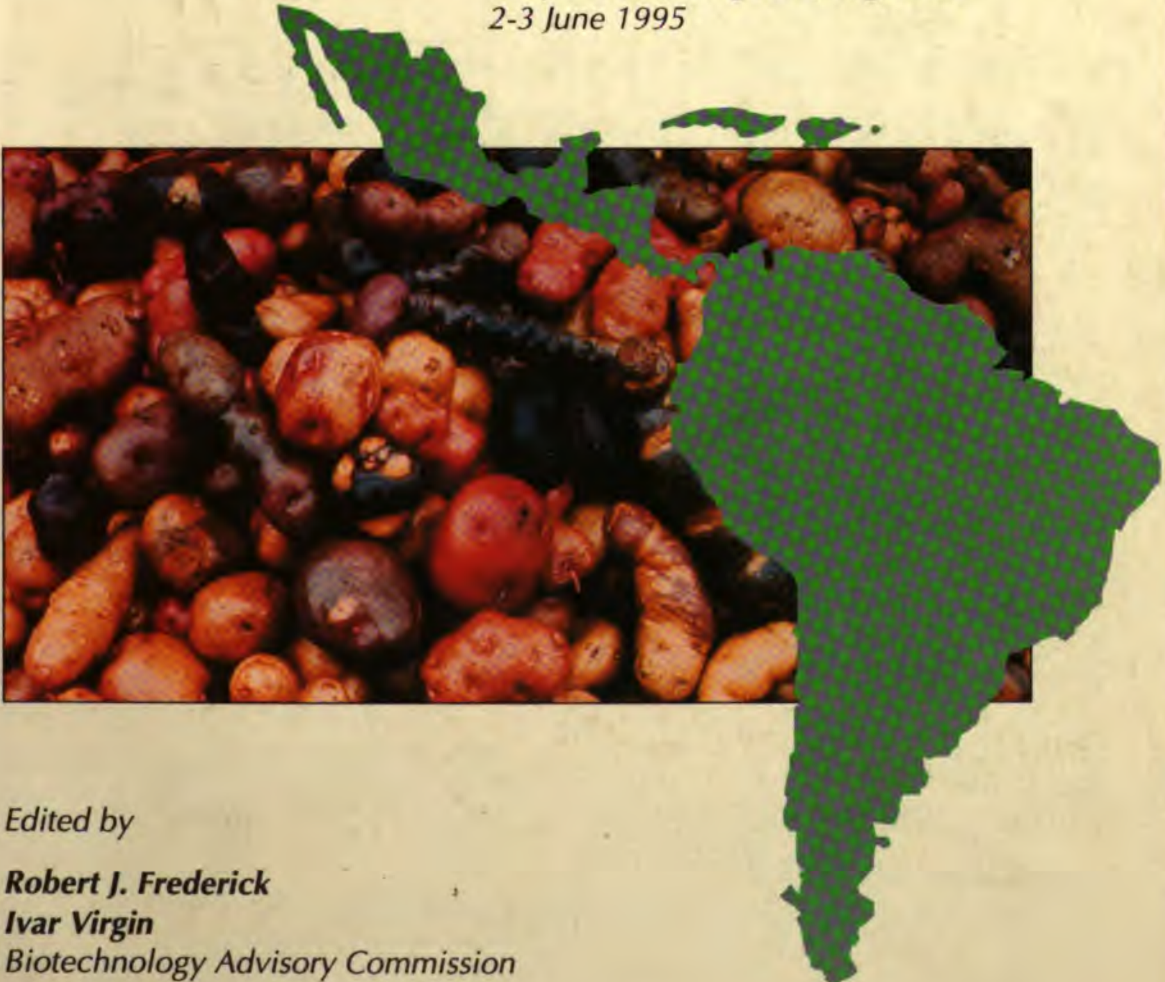
ENVIRONMENTAL CONCERNS WITH TRANSGENIC PLANTS IN CENTERS OF DIVERSITY: POTATO AS A MODEL

Proceedings from a regional workshop
Parque National Iguazu, Argentina
2-3 June 1995



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IICA

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Biotechnology Advisory Commission

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For detailed addresses, see Appendix.

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* Not present at workshop.

Preface

Agriculture today faces formidable challenges. It has to be sustainable in meeting rising demands for food over the indefinite future without compromising the ability of future generations to meet their needs. When looking back at the green revolution, growth in food production has been impressive. Yields of major cereals such as rice, maize and wheat have nearly doubled between 1970 and 1994. When considered alongside population growth, however, food production growth is less impressive. During the last two decades population growth has outstripped food production in two-thirds of the developing countries. The effect of agricultural techniques on the ecosystems is also causing concern about the sustainability of intensive farming. More and more land has come under cultivation, more and more agrochemicals are used each year, and soil erosion has reached dramatic proportions worldwide. Perhaps the most significant challenge for agricultural development in the next few decades is to meet a rapid increase in demand for food and other related agricultural products with sustainable production methods. Much of this increased output must be obtained from land with marginal inputs of water and agrochemicals.

There is no doubt that the potential contribution of biotechnology and, more specifically, genetic engineering to sustainable agriculture is truly great. It is also true that changes in agronomic practice, including the introduction of new crop varieties, deserves careful oversight and monitoring. Biologists created the first transgenic plants roughly a decade ago. Since then, development has been astounding. The United States is expected to have at least five genetically engineered crops commercialized by 1996. There were early concerns that developing countries would reap little benefit from locally generated genetically engineered crops, due to inadequate advanced research facilities. However, recent development has shown that several are rapidly beginning to adapt technological advances made in the North and elsewhere to their local needs.

In Latin America and the Caribbean, many scientists will soon be field testing transgenic plants developed at their own institutes. Research progress has been particularly rapid for the potato, one of the most valuable crops in the region. Readily adapting to different climates, from temperate regions to lowland tropics, it is a staple food for large parts of the population. However, the potato is also susceptible to a number of pests and abiotic stress factors. For many years conventional breeding programs have addressed these problems, but the loss of productivity remains significant in the region. At the same time, the heavy use of insecticides and fungicides is compromising both human health and the environment. Scientists hope that the development of transgenic potatoes will help alleviate these problems. But another important fact must be considered. Latin America and in particular the Andean region is the center of genetic diversity for potatoes: an issue directly referred to in the Convention on Biological Diversity. Unlike the United States or Europe, Latin American countries must respond to questions about the likelihood of genes spreading from transgenic potatoes to wild relatives and an uncertainty about possible impacts on genetic crop diversity.

The proceedings of the BAC/IICA workshop in Puerto Iguazu, 2-3 June 1995, attempt to address questions and environmental concerns related to the small and large-scale introductions of transgenic potatoes in their center of origin. It provides a point of departure for the dialogue between national regulators and scientists in the region as they work toward the safe introduction of transgenic potatoes in Latin America. Beyond the narrow focus of this conference, the thoughts and ideas expressed here should also contribute to deliberations on the safe application of other transgenic crops in their centers of origin.

S. Ramachandran
Chairman of the BAC
Washington, November 1995

A. Paulo M. Galvão
Director, Science and Technology, Natural Resources and Agricultural Production, IICA
San José, November 1995

Foreword

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Background

Modern biotechnology has advanced beyond the discovery stages. Its use in diverse industries is evidenced by an increasingly impressive number of commercial products. Although advances in agricultural biotechnology have lagged behind early predictions, transgenic crops are in the market place and it is clear that the pace of development will increase worldwide. After the turn of the century, food and agriculture are predicted to move from a 21% share of a 20 to 40 billion dollar a year worldwide market for biotechnology derived products to a 48% share of a 45 to 200 billion dollar a year market (Sasson, 1993). This acceleration stimulates a sense of urgency to understand the implications of these developments. The rapid pace threatens to overwhelm our ability to evaluate and assess the risks and benefits of the new technologies. For developing countries, this is somewhat compounded by the fact that transgenic fruits, vegetables and grains, created and tested elsewhere, are now reaching their shores.

Status of the technology and its regulation

The adoption and expansion of biotechnologies in Latin America have been slowed for a variety of reasons; economic, political and social (Sasson, 1993). Although slow at its initiation, the interest has been strong and there are signs of an increasing pace in the use of these technologies. This is evidenced by the number of research laboratories involved in biotechnology research and the demonstrated interest to have regulatory review mechanisms in place.

One of the oft used benchmarks for the progress of agricultural biotechnology is the number of field tests of transgenic crops done to date. While an overwhelming majority have been conducted in the North, significant numbers are now occurring in the South. In Central and South America and the Caribbean, 76 had been reported by the end of 1994 (Krattiger, 1994). However, with two exceptions, transgenic crops going to the fields in Latin America have been developed in the North. That is changing. International and national research laboratories in Latin America have begun testing transgenic plants developed by their own scientists. With at least 11 Latin American research groups involved in transgenic potato research, it is the most popular crop in development. Indeed it is one of the first transgenic crop plants developed by scientists in Latin America to be field tested.

While many of these countries have a regulatory mechanism in place, the majority do not (Virgin and Frederick, 1995). By June of 1995 only five of 16 Latin American countries (Brazil, Argentina, Chile, Costa Rica, and Mexico) and one Caribbean country (Cuba) had a review mechanism. With the assistance of the Food and Agriculture Organization of the United Nations (FAO), the Inter-American Institute for Cooperation on Agriculture (IICA) and international research centers, such as the International Potato Center (CIP) and Centro Internacional de Agricultura Tropical (CIAT), there has been an increase in awareness of the need for harmonized regulations. In Costa Rica in 1992, IICA cosponsored a meeting on biosafety. This led to the publication of Latin American biosafety guidelines. In June of 1994, an Andean Region conference was held in Cartagena to explore possible modalities for regional guidelines. In March of 1995, another regional meeting was held in Costa Rica. These efforts particularly point to the interest in using not only biotechnology but also its safe application.

Science and risk evaluation

The use of scientific knowledge and understanding in evaluation processes for biotechnology risk benefit analyses has been evolving since the late eighties. As we have become more comfortable with answers to "old" issues, "new" issues seem to keep arising. One of these emerged when interests in biotechnology and biodiversity converged. Biotechnology featured prominently in the discussions surrounding the Convention on Biological Diversity (CBD). James McNeely (1994) succinctly summarized the connection this way: *The new biotechnologies may increase the value of the world's biodiversity if they allow increased use of the genetic diversity of both wild and domesticated species, thereby increasing their economic importance. But biotechnology also poses significant ecological and economic risks that could ultimately undermine its potential contribution to the conservation of biodiversity. The introduction of any new organism poses a risk to the environment, and many of the world's known extinctions have been caused primarily by the introduction of exotic species. The release of genetically engineered organisms into the environment thus deserves the most careful oversight and monitoring.*

The CBD couples access to genetic resources with scientific cooperation and transfer of technology from developed to developing countries. In Articles [8g] and 19, developing countries' concerns for the biotechnology were clearly expressed. There was a call to examine the need for an international protocol to protect against Northern intrusion of transgenic organisms into testing grounds of the South.

Picking up this theme, Drs. Rissler and Mellon (1993) bring into focus the international implications of transgenic crops - developed in the North where related species are rare - being used commercially in the South where the center of origin or diversity of these crops exist. In their recommendations, the authors call for "a strong government program assuring risk assessment of all transgenic plants, and adequate consideration of centers of crop biodiversity here and elsewhere in the world" before a company is allowed to commercialize a crop.

Workshop objective

With commercial production of transgenic tomatoes and potatoes now a reality, it seemed an appropriate time to revisit environmental concerns and to do so with the perspective of scientists from countries located in centers of origin or diversity. Biosafety assessments of transgenic potatoes represent a special challenge to regulatory officers in Latin America . Few assessments have been done in the region where, in stark contrast to the majority of requests for field authorizations being made today, the "center of origin" issue is pertinent. For this reason, transgenic potatoes in Latin America was chosen as the focus of the workshop.

Relevant topical workshops have been held in the past. One of the most significant was convened in St. Andrews, Scotland in 1991 (USDA, 1991). This workshop titled Safeguards for Planned Introduction of Transgenic Potatoes provided a public forum to present and debate the issues of concern. There, participants discussed the potential for transfer of genes from transgenic potatoes into other species or lines, the consequences of transfer, and how to eliminate or minimize adverse consequences due to gene flow. A majority of the workshop members concluded that molecular biological improvements of the potato should proceed; experiments should be designed to be as safe as possible; there is a need for careful, rational assessment of the safety of experiments and adequate funding to provide for such experiments; and there is a necessity "to demonstrate to the public that the experiments... are going to be safe." Many felt "that the only chance... to limit the spread of a gene is in the small scale experiments." The focus was on confined "experimental" releases with a northern perspective. It is important to note that the conclusions from this workshop were to a large extent based on the exclusion of gene transfer from transgenic potatoes to interfertile species due to the lack of wild relatives in USA and Europe. For introductions in a crop's center of origin, the potential impact from gene flow deserves to be considered in much more depth.

The BAC/IICA Workshop in Puerto Iguazu, was designed to take advantage of new information gained since 1991 and to consider the large-scale (commercial) stage of crop development. Potato research scientists from Latin America and invited resource persons came together to discuss the issues. The 35 attendees came from Argentina, Bolivia, Brazil, Chile, Colombia, Cuba, Mexico, Peru, Uruguay, the United States, and Venezuela. They work at universities, government agencies, non-governmental organizations, international laboratories and the private sector. A list of participants is included as Appendix. These workshop proceedings are offered in the hope that they will prove helpful, not only to participants, but to anyone with an interest in environmental concerns for transgenic plants in centers of origin.

The workshop proceedings are summarized in eight chapters. The first chapter gives an overview of the history of the potato as well as the current trends for development of transgenic potatoes. Chapter 2 reveals the current status of Latin American research on transgenic plants and in particular the development of transgenic potatoes. Chapter 3 considers the potential for and the environmental implication of gene flow from transgenic potatoes to their wild relatives. In Chapter 4 the general view on environmental concerns with transgenic potatoes are examined. Chapter 5 explores the concerns *vis-à-vis* introduction of specific transgenic traits conferring increased virus and insect resistance. Chapter 6 summarizes the views of the participants on environmental concerns and potential benefits of transgenic potatoes as expressed in special work group sessions. In Chapter 7, a report of field test data used in the United States is presented for comparative purposes. Finally, the participants' recommendations are listed in Chapter 8. Each presentation was followed by intensive discussions and we have tried to reflect and capture the essence of the debate in the discussion boxes which are assigned to appropriate chapters throughout the volume. The questions (Q) were all spontaneous in nature and, where redundancies occurred, have been edited to minimize repetition and improve clarity. For the same reasons, the responses (R) were also edited.[1]

[1] A frank and open exchange of information was encouraged during the workshop. The proceedings are the result of the combined efforts and comments of all participants. While identifying areas of broad as well as specific agreement, the report also incorporates a range of individual impressions and opinions. The opinions and views expressed in this publication do not necessarily reflect those of the Biotechnology Advisory Commission (BAC) or the Inter-American Institute for Cooperation on Agriculture (IICA). Moreover, the opinions expressed in the discussion boxes do not necessarily reflect the views of the author of that Chapter.

Limitations

While the workshop was very productive, it is important to recognize shortcomings; those intended and not. With limitations in space and financial resources, it was necessary to curtail attendance. Consequently, not all disciplines or affiliations could be represented. During the meeting for example, special note was made regarding the need for ecologists when discussing environmental impacts. The social implications of biotechnology expansion were not discussed. It should be no surprise therefore that the organizers and participants viewed this meeting as a starting point to explore the important issues of biosafety, biodiversity conservation and the potential impacts of commercial production of transgenic crops in centers of origin and/or diversity. Clearly more thought and research will be necessary to resolve many of the issues raised. It is hoped that recommendations from the meeting will encourage further discussion and debate.

Acknowledgements

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Chapter 1
Diffusion of Potato Germplasm and Modern Breeding

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Summary

This paper reviews the history of potatoes as a crop and the current trends for development of transgenic potatoes. The potato began its journey from its center of origin as an unappreciated and alien plant. Acceptance was slow to develop and almost certainly involved genetic change from the form in which it was originally introduced. It was adopted as a food of the masses, providing a reliable and usually highly productive food source. Infusions of genes from the center of origin remained rare and mostly ineffectual until the twentieth century. Our knowledge of potato and its relatives in its original habitat remained as impoverished as potato's actual genetic diversity over several centuries. In the last half century, a rich understanding has developed of the biodiversity of native potato cultivars and wild species, categories which encompass thousands of genetically unique entities. Today, the potato continues to be enhanced by extensive use of plant introductions in breeding of new varieties. Gene insertion offers new opportunities to develop varieties that will fit into a particular market niche. It has yet to be seen whether gene insertion is a major revolution or a valuable adjunct to traditional breeding. Experience teaches us that we have much left to learn and, hopefully, much benefit to reap in the future.

Initial contact

Any consideration of the genetic history of the potato must start with the observation that the origin of the potato is in South America. In 1532, when the Spanish arrived in what is modern day Peru, they saw that the potato was a major crop underlying the Inca and other civilizations of the central Andean Cordillera. Although there is no specific report of potato's introduction to Europe, the date 1570 seems likely, as there are records of a hospital buying potatoes for food in Seville, Spain, in 1573 (Hawkes, 1990).

The importance of potato as a food in the Andes seems not to have been communicated to herbalists or botanists throughout Europe. From Spain, the potato was taken to Italy, then England in 1586, and then Germany in 1601. Clusius, a Belgian botanist, received two tubers while in Vienna in 1588, and the following year received a watercolor painting, the first pictorial representation of the plant in the Old World. In England, the herbalist John Gerard included potato in a catalog in 1596 (Hawkes, 1990). Due to its adaptation to short day length, the introduced potatoes tuberized late and would only produce small tubers in environments with exceptionally long frost-free growing seasons. Consequently, for many years, the potato's potential as a food crop must have been unimpressive and it was regarded as a botanical novelty.

Botanists of sixteenth century Europe struggled to place potato in its proper category in this pre-Linnean world. Its similarity to the truffle caused herbalists to call it by that name for a time. It was not clear whether the small green fruit or the paltry tuber was intended as the edible harvested product. Those who correctly recognized its similarity to nightshades suspected it of being poisonous. Kinship with the tomato and the phallic shape of the tuber was reason enough to label it as an aphrodisiac, casting shame upon anyone who showed interest in it. It was even accused of causing leprosy, due to the appearance of the skin.

Adoption as a food

Initial ambivalence and outright opposition were converted, slowly, during the course of a century and a half, into the extensive use of the potato as an important part of the diet in certain geographic areas. How this might have occurred is subject to speculation, but it is known that the initial introductions bore fruit, containing seeds of sexually produced progeny. These could have been selected for greater adaptation to long days, a process which could have occurred unconsciously in the hands of gardeners. In any case, the increased yield would have led to recognition of potato as a possible staple in the diet. By the late eighteenth century, the potato was identified in France as a famine food, which reliably fed masses when other crops failed. It was promoted in great degree by Antoine-Augustin Parmentier, who had first eaten it as a prisoner of war in Germany. Later he wrote several tracts (1773 through 1786) describing the chemical composition, methods of cooking, and horticultural practices. His essay, "Research on Nourishing Vegetables to Substitute for the Usual Foods During Famines," extolled the adoption of the potato as necessary for stability of food supply and cultural progress in France (Parmentier, 1781).

There are a number of attributes of the potato which permitted it to find a unique niche in the plant husbandry of Europe. The tuber seed was relatively large, providing for a vigorous initial growth phase, and, if severe adversity was encountered, abundant re-emergence from new sprouts. Production on marginal soils was substantially greater than for wheat or barley. The potato gave half or a third of a crop under conditions that would lead to total crop failure for small grains. The passage of an army, either friend or foe, often led to the confiscation or destruction of food in any form, a harbinger of famine. Naturally, the subterranean location of the potato frequently protected it from pilferage. A wheat field near harvest could be burned or livestock stolen, but potatoes were out of sight. Potatoes required no processing other than cooking, which could be done as simply as placing them in the embers of a fire. The ease of preparation converted the householder into a self-sufficient provider of sustenance from sowing of the crop to ingestion of the harvest. Thus, in spite of its alien nature, the potato became an important crop of the masses.

The potato in Ireland

Potato became supremely important in Ireland. Ireland of the eighteenth and nineteenth centuries was an economic colony of England. Most Irish families lived as tenants on land owned by English landlords. In return for tenancy, they were expected to produce meat and grains, which were to be exported to provide income for the landlord. The potato entered this situation as a provider of food for the household. Since it was not part of the tenancy relationship, potato was grown on the poorest soils, yet yielded well when planted on "lazybeds," raised strips of soil, fertilized with animal manures (Salaman, 1985). The carryover in the soil of unharvested tubers (volunteers) often meant that replanting was unnecessary. Since the Irish were trapped in an impoverished economic servitude, the appearance of a foodstuff that could be solely their own and of no interest to the landlord was mostly a blessing. The potato's merits coincided with, and may have been a causal factor in, a population explosion. The population of the Irish increased from 1.5 million to nine million between 1790 and 1845 (Hobhouse, 1986).

Early concepts of potato breeding

Despite the fact that scientists of the day knew where it had originated, only a few, if any, new potato introductions had occurred up until two and a half centuries after introduction. The few types that appeared were difficult to maintain because they produced tubers late in the season, and they became infected with virus and were lost. The value of breeding earlier maturity in varieties was recognized and attempted by selecting sexual progeny. Furthermore, all varieties appeared to decline in yield, and replacement of old clonal cultivars with new sexually produced seedlings was a method of restoring vigor. Yet potato breeding was basically recycling a static gene pool (Glendinning, 1983).

The narrow genetic base meant the potato was vulnerable. In 1845, late blight disease, incited by the fungus *Phytophthora infestans*, appeared in Ireland. The varieties in Ireland, foremost among these the variety "Lumper," had no resistance and failed in the field or rotted in storage. There were few options as the Irish lived at subsistence levels and the economy was controlled by English landowners. They lived virtually outside a cash economy, since they were not paid anything for their produce, but rather supplied it as payment for tenancy. Even during the famine, small grain stocks were exported because the landlords could reap tremendous profits due to a worldwide food shortage. The Irish had no money to buy food belonging to their landlords. The utter dependence of the Irish diet resulted in massive starvation and emigration. It is estimated that a million people starved and two million emigrated.

Goodrich's introduction, "Rough Purple Chili"

The late blight epidemic was the first example of widespread crop failure due to a narrow genetic base. Late blight heightened awareness of the need to develop resistant cultivars. The Reverend Chauncey Goodrich of New York State obtained potato clones of South American origin through the Consulate in Panama in 1851. With hindsight, one may speculate that the potatoes originally came from Chile, where the day length would be similar to New York. The single clone that he kept out of these introductions was named "Rough Purple Chili." Successive sexual cycles stemming from seedlings grown from open-pollinated seedballs led to "Garnet Chili" and "Early Rose." Goodrich saw the potato as healthful food, which could uplift humanity if varieties of sufficient hardiness could be bred. To him, potato breeding had more the character of a crusade than a hobby. Working with germplasm introduced from the area of origin was difficult. He discarded all but a few clones after considerable labor over many years, doubting whether even these were worthy. Although Reverend Goodrich believed his efforts were a failure, the impact of his breeding has been tremendous due the use of "Early Rose" as a universal parent. "Russet Burbank," the most important processing potato in North America, is a somatic mutant of "Burbank," which was a progeny of "Early Rose". Thus, "Rough Purple Chili" was a great-grandparent of "Russet Burbank," and an ancestor to more than 100 North American varieties and more than 300 European varieties (Hawkes, 1979; and Plaisted and Hoopes, 1989). On the other side of the Atlantic, a counterpart of sorts to Chauncey Goodrich, William Paterson of Dundee, Scotland, commenced breeding efforts in 1853 with the express purpose of obtaining greater blight resistance. Although it is debatable if blight resistance was obtained, one of his varieties, "Paterson's

Victoria," was a great success and over time became an ancestor to the most important varieties of the early twentieth century (Salaman, 1926).

The last quarter of the nineteenth century was characterized by considerable private interest in potato breeding. Generally, breeders only made crosses between established varieties. As a consequence, the nature of the gene pool changed very little. Breeders did not keep breeding lines that might have had important characters yet lacked sufficient performance for commercial success. The origin of varieties was often a secret, and there were cases of varieties being re-introduced under new names for profit motives (Glendinning, 1983).

Use of *Solanum demissum* for resistance to late blight

An important breakthrough in resistance to late blight was discovered in the Edinburgh Botanical Garden in 1910. The entire tuber-bearing collection was killed by late blight with the exception of one accession from Mexico. This accession was called *S.* x *edinense*. Later, it was determined that it was a hybrid of cultivated potato with the wild Mexican species *S. demissum*. Late blight resistant breeding lines were developed in Germany and the United States, and constituted the first intensive use of a wild species to achieve a specific improvement of a trait in potato breeding history.

Systematic germplasm collection

Starting in 1925, scientifically planned collecting expeditions were conducted beginning with Soviet scientists under the guidance of Vavilov. Usually collections were restricted to certain geographic areas. Wild species and native cultivars were collected. Expeditions sponsored by the Soviet Union were conducted in 1925-6 and 1927 by Bukasov and Juzepczuk, respectively. In 1930, an expedition sponsored by Germany was conducted by the team of Bauer and Schick, followed by an expedition led by Vavilov from the Soviet Union in 1932. These were followed by an expedition sponsored by Sweden in 1933-4 conducted by Hammarlund and two expeditions in 1938 and 1939 sponsored by Great Britain. These were carried out by Balls and Gourlay in the first trip, and the team Balls, Gourlay, and Hawkes, on the second expedition. Professor Hawkes has, over more than a half century, literally unlocked the secrets of the tuber-bearing *Solanum*. These expeditions led to the establishment of germplasm banks in Europe and the United States and the synthesis of the first cohesive taxonomy of the wild relatives of potato. Evaluation of entries in these banks for resistance to pests and pathogens led to the intensive utilization of certain wild species as sources of resistance. By the mid-twentieth century, this work was well underway in Europe. Only more recently has this been the case in the United States and Canada. About this time, it was realized that the late blight resistance from *S. demissum* was not durable. There was a gene-for-gene correspondence between the potato host resistance and *Phytophthora* pathogen virulence. Blight immune cultivars developed from *S. demissum* become completely susceptible when a new virulence genotype of the fungus appears. Since 1950, there has been a general search for durable sources of resistance to *Phytophthora*. This implies that resistance is based on multigenic impedance of infection and disease progression at several points in the pathogenesis process (Toxopeus, 1964).

Extensive collection in the last 50 years has permitted the elaboration of a considerable body of biosystematic knowledge. Furthermore, awareness of the diversity of native cultivars has expanded. It is known, for example, that eight species of potato are cultivated. There are about 5,000 cultivars maintained in the collection at the International Potato Center (International Potato Center, 1988). The vast majority of cultivars are classified as *Solanum tuberosum* ssp *andigena*, a tetraploid. The second largest group are the diploids. Triploids and pentaploids are much less numerous, and two of these, *S.*x *juzepczukii* and *S.* x *curtilobum*, are specialized as frost resistant cultivars by virtue of being hybrids with the wild species *S. acaule*. These cultivars are grown at the very highest elevations of potato culture. They are high in glycoalkaloid content, requiring processing to remove these bitter compounds to make the edible dried product *chuño*. Bitter potatoes are still grown today but lack high value in the marketplace and are therefore restricted to frost-prone environments. *Chuño* captured the attention of the writers of the first commentaries, the early Spanish conquerors, for their storability, transportability, nutrition, and availability during famine caused by crop failure.

Discussion:

Q: *Should not the existence of large* ex-situ *collections of* Solanum *species diminish the concerns for loss of diversity?*

R: Only 50 percent of wild *Solanum* species are in *ex-situ* collections. Continuous *in-situ* evolution of wild *Solanum* species may be more valuable than *ex-situ* conservation.

Native cultivars in modern times

In modern times, native cultivars are found primarily in rural locations. To a certain extent, they are in competition with modern day bred varieties yet their appeal is still retained because they are prized for their culinary traits. Thus a rural household may grow a bred variety, *papa aguanosa* (watery potato) or *papa blanca* (white potato), for sale to city markets because the yields are higher, whereas the old traditional varieties, *papa de regalo* (gift potato) or *papa de color* (colored potato), will be retained for autoconsumption or sale of surplus in the local markets, usually at premium prices.

A detailed examination of two mixed fields in southern Peru identified 26 distinct varieties, five of which were diploids, three triploids (not bitter types), and the remainder tetraploids (*Solanum tuberosum* ssp. *andigena*) (Jackson, Hawkes, and Rowe, 1980; Quiros, et al., 1990). Another study near Cusco in southern Peru found that a field with native traditional cultivars may have from 10 to 30 varieties, averaging 20. Out of 28 mixed potato fields sampled, 79 cultivars, which could be further separated into 164 subcultivars, were distinguished. Out of the 79 cultivars, one-third were cosmopolitan in being found across three distinct regions, whereas the rest were restricted in distribution, and in certain cases were limited to only one or two fields over the whole sampling area (Zimmerer, 1991).

Potato folk-nomenclature reflects considerable sophistication in differentiation by morphology and use in the household economy. Comparisons of biochemical genetic markers and local names indicate a high degree of correspondence between farmer identification and genotype, although occasionally similar appearing but genetically different varieties are classed as one variety by farmers (Quiros, et al, 1990). Potato varieties are classified according to their method of preparation as being more suitable for boiling, soups, mashed potatoes, or for yellow flesh dishes; or bitter potatoes for processing into *chuño,* made from frost resistant cultivars. Cultivar names, often in the *Quechua* or *Aymara* languages, are descriptive of: appearance - "cat's face," "black girl," "llama's tongue," "puma's paw;" or function "potato for fever" or "potato for weaning of children from mother's milk" (Brush, 1980; and Christiansen, 1967).

Wild species

While there are eight cultivated species, there are approximately 200 wild species, ranging in distribution from the southwestern United States to the southern South American countries of Argentina and Chile. Collections brought back by expedition participants in the form of living tubers or botanical seeds provided pathologists, nematologists, and entomologists an opportunity to screen for resistance. It has been discovered that many of these species have very high resistance to pests and pathogens. This has led to the modern day incorporation of wild species genes into numerous varieties. Examples of this are resistance to *Globodera* (potato cyst) nematode derived from *S. vernei* and other species (Ellenby, 1948; Ellenby, 1954; Mai, and Petersen, 1952), resistance to potato virus X from *S. acaule* (Ross, 1954), and resistance to potato virus Y from *S. stoloniferum* (Ross, 1958). Resistance to late blight was derived from *S. demissum* as already described. In addition, there are promising research programs where resistances to other pathogens or pests may become incorporated into potato varieties in the future.

Utilization of exotic germplasm

There has also been interest and intensive activity to bring in short-day adapted native cultivar germplasm to enrich the diversity of the long-day adapted gene pool of the higher latitudes. This has involved the use of diploid cultivated material in conjunction with diploid wild species by use of interploidy crosses (De Jong, et al., 1981; Hougas and Peloquin, 1958; Iwanaga, 1984; and Peloquin, 1982), and the direct use of spp. *andigena* in breeding programs (Glendinning, 1975; Rasco, et al., 1980; and Tarn and Tai, 1977).

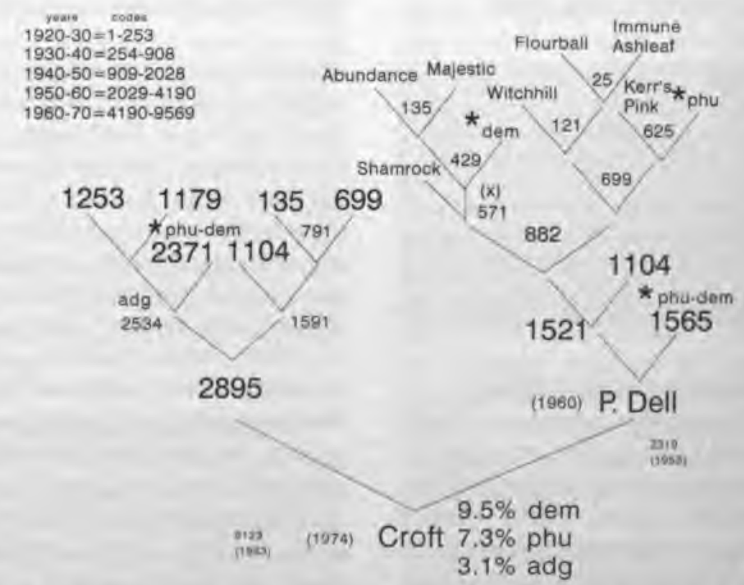

Figure 1. Pedigree of "Croft," a recent variety. Numbered codes are keyed to years during which the selection was performed. Symbols are as follows: dem= *S. demissum*; adg= *S. tuberosum* ssp *andigena*; phu= *S. phureja*; (x)= selfing; P. Dell= "Pentland Dell"; *= cross where exotic germplasm was used. Specific dates when the cross was made or the variety released are in parentheses. Excerpted from Glendinning (1983).

Modern breeding is taking advantage of the germplasm resources available from the center of origin of potato. A detailed pedigree of the variety "Croft" is shown in Figure 1. The use of only named varieties as the distant ancestors of "Pentland Dell" reflects the breeding practices of the late nineteenth and early twentieth centuries. The native cultivars *S. phureja* and *S. tuberosum* ssp *andigena* were used as well as the wild species *S. demissum*. The genetic composition of "Croft" consists of 20% exotic germplasm. In Figure 2 the origin of the variety "Raritan" is shown. The pedigree shows the use of a single variety, "Katahdin," as a recurrent parent into which the late blight resistance derived from *S. demissum* was backcrossed. It was unusual to use a single backcross parent because this introduces inbreeding into the breeding population. The targeted goal of introducing a single trait from an exotic source has been carried out numerous times in potato breeding history.

Gene technology

The advent of technological means to insert foreign genes in potato has increased the options for potato variety development. There are two advantages that have accrued. First, it has been possible to add traits to popular varieties. The fact that it is virtually impossible to recover parental phenotype in sexual breeding of potato lends great appeal to this feature of gene insertion. A second benefit is the enlargement of genetic variation beyond that which is available within *Solanum* spp.

The outline in Table 1 attempts to inventory in a partial fashion the types of genes that have been introduced into potato.

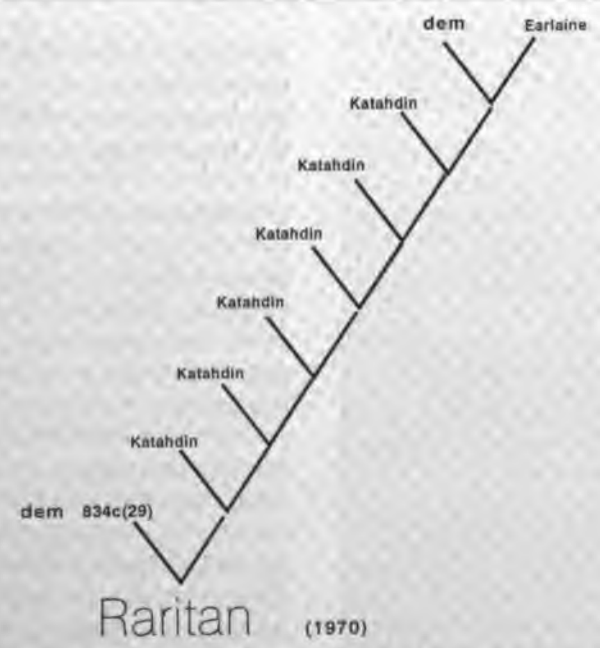

Figure 2. Pedigree of "Raritan," a variety derived by backcrossing late blight resistance trait from *S. demissum* into a cultivated background using "Katahdin" as the recurrent parent. The practice of using a single recurrent parent is unusual, but the introgression of traits from exotic species has occurred in many instances.

TABLE 1: TARGETS FOR GENETIC MANIPULATION IN THE POTATO

Traits	Genes
1. Fungal resistance	a. glucanase gene b. chitinase gene c. osmitin gene
2. Bacterial resistance	a. cecropin gene b. lysozyme gene
3. Insect resistance	a. *Bt* toxin genes i. *Cry* III for *coleopteran* resistance ii. *Cry* I genes for *lepidopteran* resistance
4. Herbicide resistance	
5. Virus resistance	a. coat protein genes b. replicase genes c. non-translatable modified versions of coat protein genes
6. Altered starch content	a. high starch genes
7. Stress resistance	a. drought tolerance genes b. frost tolerance genes
8. Anti-bruise genes	a. suppression of polyphenol oxidase b. scavenging of tyrosine.

There is one further comment that defines an essential difference that gene insertion has introduced to the genetics of potato breeding.

Discussion:

Q: Is there some definitive evaluation regarding the potential loss of crop diversity versus an increase of diversity based on the ability to work with new genes? Can we determine the impact on breeders in this kind of effort?

R: Biotechnology has to be recognized as a tool. While conventional breeding may become more precise, it will not be replaced. Economics certainly are a consideration here. Relative to the entire gene pool, very few genes are being worked on to establish attractive traits in transgenic crops.

Q: If specific gene constructs outcross to the wild Solanum *populations and are recovered in other breeding programs,can the gene be used subsequently for commercial purposes?*

R: This situation is not of high concern in the United States where restrictions on property rights protect companies from the use of constructs developed by others. (No comments were offered on the situation in Latin America.)

While traditional breeding relied upon crossing and backcrossing to introduce new traits to the breeding pool, gene technology introduces one or a few genes that are expressed dominantly to achieve a phenotype. When one is considering the effect that gene migration from cultivated to wild potato populations might have, it is clear that the phenotype will be more efficiently transferred when single dominant genes control expression. Multigenic traits require multiple gene transfer, and as the terminology implies, the phenotype will be recovered less frequently in sexual hybrids of a recipient population. Because transgenes are always monogenic and dominant in expression, transfer of a transgenic phenotype will be of the most efficiently transferred category. With a few exceptions, characteristics of cultivated potato, non-transgenic, are multigenically controlled and very inefficiently transmitted sexually. Therefore, transgenes are in the most potent genetic category and as with any dominant monogene are likely to be expressed in undiminished form if transferred to wild populations of potato.

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Chapter 2
Current Status in South America

Editors' note: The current status of biotechnology development was very relevant to the discussion and perspective of the workshop. Accordingly, knowledgeable scientists were asked to briefly describe current research programs and regulatory procedures in their countries. This, we believe represents the most accurate and up-to-date descriptions available.

Argentina

(Esteban Hopp, Comision Nacional Asesora de Biotecnologia Agropecuaria)

Argentina has a national biosafety commission, CONABIA (Comision Nacional Asesora en Biotecnologia Agropecuaria). It was created to design a regulatory infrastructure based on the IICA guidelines and previous experience of USA, Canada, and Europe. The CONABIA is connected to the Agricultural State Secretary and has multi-disciplinary participation of scientists (i.e., from universities, agricultural research institutes, quarantine services, and seed companies). Instead of creating new regulations, Argentina harmonized the existing plant protection and seed management laws (previously developed for non transgenic plants) with biosafety regulations in other countries. The current regulations are flexible enough to allow pertinent changes derived from the experience in other countries in this rapidly evolving field. Regarding field trials, Argentina has the largest number and greatest diversity of trials performed in South America (more than 40). Among tested crop species are: maize, cotton, rapeseed (canola), soybean, tomato, sugarbeet, sunflower and wheat. The traits include: insect and virus resistance, herbicide tolerance, male sterility, oil quality, delayed maturation and marker genes. Most of the tests have been performed by foreign, Northern Hemisphere companies taking advantage of the possibility to perform off-season evaluation and seed production of the crop in the Southern Hemisphere. Currently, there are six different laboratories in Argentina working with transgenic crops; two of them on transgenic potato. Most of the work is done on virus resistance. No field trials with transgenic potatoes have been performed.

Discussion:

Note: Argentina has a review system similar to that of the USDA, at least in the terms of the kind of questions that are asked in the evaluation process. In some of the trials being carried out in Argentina, the USDA-APHIS has been asked to provide detailed information from U.S. applications. So far, forty permits have been approved and the country is close to having several transgenic crops (soybean, maize, tomato) deregulated. In order to take food to the commercialization stage in Argentina there must be a health and safety review. There are no special regulations for products at the laboratory stage. All strains originating in national laboratories will be publicly available.

Bolivia

(Miguel Angel Silva Ramos, Research and Extension Department of the Agriculture & Livestock Secretariat)

Regarding biosafety regulations, the workshop on biosafety harmonization in the Southern Cone of South America (Buenos Aires, November 1992), organized by IICA, contributed to the realization of a national seminar on biosafety and introduction of transgenic plants. This in turn led to the formation of the National Biosafety Committee created in Santa Cruz, Bolivia, in July 1993. Biosafety regulations and guidelines have been formulated and will be approved by the national authorities in 1995. Bolivia also took part in the Cartagena meeting[1] and adopted its recommendations which aimed at a harmonization of the biosafety guidelines in the Andean region. Regarding transgenic plant research, the Bolivian Institute of Agricultural Technology (IBTA) has a project on transgenic potatoes resistant to frost. The research project is a joint effort with the Central University of Venezuela (UCV), the University of Louisiana and the International Potato Center (CIP). In November 1993, transgenic potatoes from the UCV expressing antifrost proteins were evaluated in field trials performed in Bolivia. The trials were guided by biosafety regulations applied and produced at CIP as well as the National Biosafety Committee and ISNAR recommendations. No other field trials with genetically modified plants have been performed in Bolivia.

Discussion:

Q: Is there pressure from abroad on Latin American countries to use certain genetic constructs?
R: There is currently not any pressure to use specific constructs. Recognizing that the first generation of gene constructs came from the North, these have been useful to facilitate the learning of transfer techniques and to build in-country capacity. Now, several countries in Latin America are developing their own gene constructs which better reflect the true agricultural needs of the region.

Q: In which South American countries have transgenic plants been field tested?
R: Transgenic crops have gone to the field in Argentina, Chile, Peru, Bolivia, Mexico and Cuba. In all these countries trials have been subjected to regulatory oversight.

Brazil

(Luiz Barreto de Castro, Ministério da Ciência e Tecnologia)

The Congress of Brazil approved and the President of Brazil sanctioned Law No. 8974 of January 5, 1995, which establishes norms for the use of genetic engineering techniques and the release of transgenic organisms into the environment. The law also gives executive power to create the National Technical Commission of Biosafety, which is linked to the administrative structure of the Ministry of Science and Technology. By Presidential Decree No. 1520 of June 12, 1995, the competence level, administrative links and composition of the Commission were established. The Commission has representatives from the Ministries of Agriculture, Environment, Health, Education, Science and Technology and Foreign Affairs plus representatives from the scientific community, industry, and the public. It will be operative in the second semester of 1995 as soon as the law is implemented and the Commission constituted. Transgenic plants produced in Brazil and elsewhere are in line to be field tested in 1995.

Chile

(Carlos Muñoz and Loreto Holuigue, Instituto de Investigaciones Agropecuarias and Carmen Cabrera, Servicio Agricola y Ganaderio)

Chile is used as a winter nursery for various international seed companies of the Northern Hemisphere. In recent years, some of these companies have shown interest in multiplying seed of transgenic plants. To deal with these

[1] Seminar on Harmonization of Biosafety in the Americas, 7-10 June, 1994. Cartagena, Colombia.

requests and following an agreement reached among countries in the Southern Cone of South America (Buenos Aires, Nov. 1992), the Ministry of Agriculture decided to regulate trials with transgenic plants using the phytosanitary and seed production regulations already available in the Chilean legislation. Furthermore, in 1993, an advisory committee for the Servicio Agrícola y Ganaderío, the phytosanitary regulatory organization of Chile, was appointed. Members of this committee are scientists and technical staff of the Ministry of Health, research institutions and universities. Also a resolution was issued (Res. No. 1927/93) to establish conditions for the introduction to the country of seed from transgenic plants. Since the establishment of these measures, several transgenic species have been introduced and propagated in Chile for re-exportation of the plant material. Although activities in relation to the development of transgenic plants in Chile started several years ago, no field trials with transgenic plants developed in Chile have been carried out.

The potato was used as a model species for the introduction and development of the transgenic technology to the country. Early experiments were done at the Catholic University of Chile (PUC), but more applied work is now underway both at this University and at the Instituto de Investigaciones Agropecuarias (INIA). At INIA, work is aimed at the introduction of resistance to potato viruses, particularly PVX, PVY and PLRV. This project was undertaken jointly with the National Institute of Agricultural Technology (INTA), the institution that developed the transgenes. The idea of this project is to evaluate the available transformation techniques to incorporate virus resistance into cultivars released by INIA's potato breeding program. Greenhouse evaluation of the resistance was recently initiated, applying strict biosafety regulations. Another project is being carried out jointly by INIA and PUC, where PUC is developing transgenic potato and INIA is responsible for their evaluation. The objective is to find resistance to the serious, world-wide, bacterial disease caused by *Erwinia* spp. The International Potato Center (CIP) provided the transgenes which code for various lysozymes. These genetic constructs were improved and used to transform the cultivar Désirée. Presently, experiments are underway to evaluate resistance under greenhouse conditions. Work is also starting at INIA to obtain transgenic melons resistant to Watermelon Mosaic Virus II. Future work in Chile includes using other transgenes, in particular those coding for delayed ripening, resistance to insects and resistance to other fungal diseases.

Discussion:

Q: Is there pressure from North America to put regulations in place, or is motivation out of a national interest?

R: Chile is a winter cultivation site for many companies not only from North America but also Europe. While there are no specific regulations in place for experimental work beyond the greenhouse level, there is an advisory committee that, on a case by case basis, evaluates these counter-season trials. Technology development and in-country capacity will benefit from collaboration abroad and with Northern companies. It is not just a matter of Northern pressure. There is national interest in having biosafety regulations in place.

Colombia

(Rodrigo Artunduaga Sala, Instituto Colombiano Agropecuario)

A Colombian law proposal has been prepared with the participation of the Ministry of Agriculture, Forestry and Fisheries, Ministry of the Environment and the Colombian Agricultural Institute. This law proposal recommends the establishment of a National Biosafety System in Colombia, with the mandate to establish policies and procedures to govern the use of modern biotechnology. This includes the formulation of the National Biosafety Guidelines. Within Colombia the responsibilities for regulating the field testing of genetically engineered organisms will probably reside in the Colombian Agriculture Institute (ICA), when the congress approves the proposal. Biotechnology research is currently done at several private and public institutions in Colombia mainly in tissue culture, molecular characterization, protoplast isolation and related fields. The Centro Internacional de Agricultura Tropical (CIAT) has developed transgenic beans which are at the greenhouse stage. No field trials have yet been performed. At CIAT an institutional biosafety commission has been formed. At ICA limited work is done on transgenic plants; most of the work is based on conventional breeding techniques. In the case of transgenic potato, there is a major research project in its initial stage being done cooperatively between the Colombian National University, the University of Florida (USA) and ICA. The goal is to transform a commercial potato cultivar ("Pardo Pastuza") with viral coat proteins inducing resistance to viruses PVX, PVY and PRLV. This project, initiated last January, is currently at the tissue culture level. No field tests with transformed potato have been considered to date.

Cuba

(Pedro Oramas, Plant Biotechnology Division, Center for Genetic Engineering and Biotechnology)

One of the main tasks of the Plant Biotechnology Division at the Center for Genetic Engineering and Biotechnology (CIGB) in Havana has been the production of transgenic plants with increased resistance to pests and diseases. Since 1990, the CIGB has performed 13 field trials with five different transgenic plants (tobacco, sugarcane, sweetpotato, cabbage and potato) in 10 different regions of Cuba. Traits include resistance to insects (against Lepidoptera or Coleoptera) through the constitutive expression of *Bacillus thuringiensis* endotoxin genes; resistance to virus against PVX, PVY and PLRV through viral coat protein gene expression; and fungal resistance (against *Alternara solani*) through the expression of AP-24 and basic b-glucanase genes. Transgenic potato has been systematically tested under field conditions in Cuba. It served as a model to establish the regulations for environmental releases of genetically modified plants under controlled conditions. The national regulations for environmental release of genetically modified organisms (GMO) to the environment has been under revision by official authorities of the National Congress since 1993 when a National Biosafety Commission was created to design a regulatory infrastructure in order to establish conditions for the GMO releases. The commission is an integration of university and research institutions as well as scientists and authorities from the Ministries of Science, Environment and Agriculture and the National Academy of Science. The National Law on Environmental GMO release is expected to be adopted by 1996. However, CIGB releases of transgenic plants have been performed taking all appropriate safety measures to ensure protection of the environment by following the norms of the APHIS/USDA, NRC(USA), OECD, Agriculture and Agri-Food Canada and IICA.

Mexico

(Ariel Alvarez Morales, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional)

In 1989, a National Committee for Agricultural Biosafety (CNBA) was implemented in Mexico. The CNBA falls under the direction of the Plant Health Department of the Ministry of Agriculture. The Committee is composed of seven members from different government and academic institutions. The Committee analyzes all requests regarding importation, interstate transport, controlled release and deregulation of transgenic plant varieties or products. Several import and field test permits have been granted for plant material with foreign genes introduced through genetic engineering techniques. The tomato exhibiting longer shelf life (the FLAVR Savr) has been deregulated. In the case of potato, the Irapuato Unit of CINVESTAV has performed two field tests with transgenic lines showing increased resistance to potato virus X and Y (PVX and PVY). This year a third multi-site test will take place and two more tests are scheduled for 1996. These field tests are being conducted with the help of local growers and researchers from the National Institute for Forestry and Agricultural Research. It is hoped that by the end of 1996 CINVESTAV will have enough data to make a request to CNBA for deregulation of these potatoes. If granted, this will mean that these potato lines can be as freely used as any non-transgenic potato variety. Simultaneously, a request will be submitted to the Ministry of Health to obtain a permit to commercialize and distribute these products for human consumption.

Discussion:

Q: Is there a Mexican perspective on evaluating transgenic crops given the fact that many are close to being commercialized ?

R: There has been limited reflection on the issue of containment in Mexico because requests from private companies for field testing came at a time when they were close to commercialization stage in other countries. At this point in product development there will not be containment. Consequently, in Mexico there is a greater emphasis on evaluation of potential effects of genes. The Mexican authorities have been asking for information generated in the United States which subsequently has been used in their own evaluation process.

Peru

(Ali Golmirzaie, Luis Ñopo, and Marc Ghislain, International Potato Center (CIP), Genetic Resources Department)

Potato production involves large quantities of pesticides to assure high yields. Farmers and the environment are directly in contact with these toxic chemicals. At CIP, the production of transgenic plants is conceived to increase pest and disease resistance to reduce pesticide uses in potato crop management. Resistance to Potato Tuber Moth has been successfully engineered using specific toxins produced by a *Bacillus thuringiensis* gene. Attempts to use protease inhibitors are also underway.

Transgenic potatoes resistant to bacterial diseases, such as soft rot and bacterial wilt, are engineered using proteins responsible for bacterial membrane lysis. These lytic peptides from the giant silk moth (cecropin B and attacin E) and from chicken or phage have been tried, but no significant field resistance has been observed. Virus resistance is engineered using the viral coat protein of PVX, PVY, and PLRV. For PLRV, first results reveal partial resistance of some transgenic potato lines. Viroid resistance is engineered using a pseudo-antisense approach. A delay in the disease appearance has been observed in some transgenic potato lines. Antifreeze protein genes from a polar fish have been used to induce frost tolerance in the Andes. CIP operates through research contracts with scientists in the public and private sectors that own these valuable genes. Ready-for-use gene constructs are often made available to the center. CIP produces transgenic potatoes choosing genotypes relevant to developing countries. Transgenic plant evaluations (biosafety assessments) are continuously made as work proceeds from laboratory to greenhouse and eventually to field trials made at specific CIP research stations. The process complies with the biosafety regulations existing at CIP and in the countries where field trials are to be conducted. Up until June 1995, there have been three field trials made by CIP in Peru or in neighboring Andean countries. The CIP guidelines have been adopted by Peruvian authorities as the national biosafety regulation for the introduction of genetically modified organisms into the environment.

> **Discussion:**
>
> *Q: How has the evaluation of trials prior to going to the field evolved in Peru?*
> R: The process started with the development and institutionalization of the CIP Biosafety Guidelines. These were used at the institution for an internal review process with cooperation from national officials. The Peruvian authorities gave approval prior to the field trials.
>
> *Q: How has CIP treated the experimental plots after the field trials?*
> R: All tubers were collected on the approximate half-hectare area which was left fallow for a year. All organic material was destroyed by burning.
>
> *Q: In planning field trial monitoring programs, was any consideration given to post-trial observations to substantiate or refine* a priori *decisions based on laboratory and greenhouse information?*
> R: Scientists at CIP are interested in doing this although it has not been extensively investigated. There has been some published work (see Chapter 3 this volume) on surveys to quantify pollen transfer around test plots and monitor subsequent gene dispersal.

Uruguay

(Daniel Pagliano, Instituto Nacional de Investigacion Agropecuaria)

Regarding biosafety regulations, the importance of regional harmonization of biosafety regulations has been officially expressed. In this respect, the Instituto Nacional de Investigacion Agropecuaria (INIA) maintains surveillance within the field of biosafety and is coordinating activities and information with other institutions in Uruguay. At this moment, there are no specific biosafety regulations for Uruguay. The genetic engineering research in Uruguay has primarily focused on the development of transgenic potatoes and vegetable fodder. In the case of potato, research at the laboratory level has been carried out at INIA using genes encoding for virus-resistance. Through international collaboration, transgenic potatoes resistant to the viruses PVX, PVY and PLRV are being produced. In the case of vegetable fodder, research has been carried out on the leguminous *Lotus Corniculatus*, cv. *San Gabriel*, a fodder species of regional importance. As a result, greenhouse tests with transgenic legumes resistant to herbicides have been performed and evaluated. So far, no field trials with transgenic organisms have been carried out in Uruguay.

> **Discussion:**
>
> *Q: Have transgenic plants been evaluated in Uruguay?*
> *R:* The Ministry of Agriculture has not received any formal requests for introducing or field testing a transgenic plant in the country. In a public discussion on transgenic crops and safety issues, farmers were more concerned with how soon they would be available to them than with the transgenic nature of the crop itself.

Venezuela

(Eva de Garcia, Laboratorio de Biotecnología Vegetal, Centro de Botánica Tropical, Universidad Central de Venezuela and Eduardo Lindarte, IICA-Venezuela)

In general the country has had little experience with research involving transgenic organisms. The best known project is at the Laboratorio de Biotecnología Vegetal at the Centro de Botánica Tropical, Universidad Central de Venezuela, where potato cv Désirée clones, obtained from the International Potato Center (CIP), have been transformed with the gene coding for an antifreeze protein from a polar fish. In 1991, transgenic microtubers were sent to IBTA-PROINPA in Bolivia, where the transgenic clones were tested for frost resistance. Seven clones tolerant to frost and with high yield were selected for further studies at the Laboratorio de Biotecnología Vegetal. Research is also carried out at the Universidad de Mérida on transgenic papaya (*Carica papaya*) conferring increased virus resistance. No specific biosafety norms address the subject legally other than indirect provisions contained in the existing Plant Protection Law and in a Ministerial Resolution on *in vitro* propagation of banana. In 1995, a new Law on Seeds and Plant Breeding (understood to cover any plant structure of a botanical species used for sexual or asexual reproduction) was enacted but its main objective is to ensure intellectual property rights. However, Venezuela took part in the Cartagena Accord-Andes Pact meeting[2] and adopted its recommendations which aimed at a harmonization of the biosafety guidelines in the Andean region.

[2] Seminar on Harmonization of Biosafety in the Americas, 7-10 June, 1994. Cartagena, Colombia.

Chapter 3
Ecology and Reproductive Biology of Potato: the Potential for and the Environmental Implications of Gene Spread[1]

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Summary
The use of transgenic plants in commercial agriculture is a reality. The traits given most interest are insect, viral and, herbicide resistance, fruit firmness, protein or oil composition and altered flower color. Field testing of transgenic plants has intensified worldwide, with potato being the most frequently tested crop. The greatest concern is the potential for the transgene to escape into natural populations and contribute to weediness or itself become a new weed. In potato, with its over 200 species, existing as a polyploid series from diploids to hexaploids, the chance for introgression of transgenes is great. In fact, it seems a certainty that it will occur. So, the question is not will it occur, but to decide what, if any, containment measures should be used. Containment decisions depend on the species and the pollinators present to promote gene flow in the proposed areas of introduction. It is essential to thoroughly consider the role of 2n gametes, Endosperm Balance Number, and stylar barriers to interspecific gene transfer. Physical barriers such as distance and trap crops can be used, as can male sterility as a genetic barrier. In the end, this decision will be driven by political and economic concerns and pressures, which may override scientific considerations and the preservation of natural gene pools.

Introduction
The use of transgenic plants in agriculture is a reality and we are just beginning to see them used commercially in the United States. It is no longer a question of whether they will be a part of our agriculture, but rather of how important they will become in agriculture, and most importantly, will they be accepted by the public. As we move closer to the time that they become a part of commercial agriculture, the biosafety debate will intensify. We may find that, while the debate was intense before their introduction, the population may become used to the idea and interest may wane. This does not mean that we should not be concerned.

Genes which have been of interest in transgenic plants are those for *Bacillus thuringiensis* endotoxins, viral coat protein genes, herbicide resistance genes and those affecting altered flower color, fruit firmness, and protein or oil composition (NRC, 1989). The field testing of transgenic plants has gone to advanced stages in the United States though not to commercial field production, except for tomato

[1] Cooperative investigation of the U. S. Department of Agriculture, Agriculture Research Service and the Wisconsin Experiment Station.

(Ahl-Goy and Duesing, 1995). Thirty-eight different plant species have been field tested to date. Potatoes, oilseed rape, maize and tomato collectively account for 60% of all testing of genetically modified plants in the world. From 1986-1993 over 1025 field trials with genetically modified plants were conducted in 32 countries. Central and South America account for 6% of all trials since 1991. They principally are seen as alternate season locations for materials being tested in the Northern Hemisphere. Since 1992, North America has led in the number of field trials, accounting for slightly more than half of the total worldwide. The number of genetically modified plant trials has increased each year since 1986. The United States had the most trials during this period with 385, followed by France with 138 and Canada with 119. Argentina and Chile led the South American countries during this period with 21 and 18, respectively. Maize, cotton, potato, and soybeans are the most frequently tested crops in the US and Central America, flax and oilseed rape in Canada, sugarbeet and oilseed rape in northern Europe. Herbicide resistance is the most commonly evaluated trait (34% of all field trials). At least through 1993, potato was the most frequently tested crop and had the highest number of engineered properties (36) including modified starch content and addition of various insecticidal and viral coat proteins. Potato trials represented 38% of the North American trials, 51% of the European trials, and 2% of the Central and South American trials. The five leading crops are: potato (19%), oilseed rape (18%), tobacco (13%), maize (12%), and tomato (10%). The private sector accounts for 71% of all trials worldwide, and they conduct more trials and involve more locations than the public sector. Eighty-three percent of the trials in Central and South America are from the private sector. Seven genetically modified plants have been approved for unrestricted trials worldwide: a herbicide tolerant tobacco in Europe,a herbicide tolerant flax in Canada, and in the United States, tomatoes with delayed ripening, oilseed rape with modified oil, virus resistant squash, and herbicide tolerant cotton and soybean (Ahl-Goy and Duesing,1995).

Risk assessment perspective and biological complexity

Dale et al. (1993) describe the advances in recombinant DNA methods and transformation procedures. They discuss the importance of transgene copy number, position, expression, stability, pleiotropy, selectable marker genes and somaclonal variation. They also express the need for considering strategies to balance the effects of the introduction of a transgene. It could upset the natural community it may enter. We could be contending with herbicide resistant weeds that we will need to find other ways to control, or insects with multiple resistances to the genes that have been introduced that we will need other tools to control. They discuss the necessity of carrying out risk assessment before the release occurs. The introduction of transgenic plants could lead to a significant investment of time and funds to satisfy the questions that could be posed. So the need for a clear risk assessment structure seems to be warranted from a regulatory standpoint as well as that exercized by the scientists doing the work.

Even though transgenes may be introduced into an organism, this does not mean that they will be expressed or even retain their expression. It is clear that transgenes are subject to silencing (Finnegan and McElroy, 1994). The host genome seems to have a way of checking itself and recognizing the presence of an alien gene and silencing it, most commonly by methylation. As little as "...90 base pairs of homology within the promoter region is sufficient to cause trans-inactivation." Transgene inactivation "...occurs at highest frequency when multiple copies of a gene are integrated either at a single insertion site or when dispersed throughout the genome" (Finnegan and McElroy, 1994). Co-suppression also is known to occur in transgenic plants. This is where the introduction of extra copies of a foreign gene to raise expression may result in the coordinate silencing of the transgene and the endogenous gene. This phenomenon is not associated with methylation and is reversible. While these problems may not be of direct interest here, they are a part of the expression of the transgenes, should they become integrated into natural populations.

Ecological questions

The United States National Research Council (1989) stated: *Two closely related ecological questions that may be important to the introduction of genetically modified plants are (1) does hybridization between crops and their wild relatives result in transfer of traits from the cultivated form to the wild relative, and (2) does such gene flow increase the weediness of wild relatives? If the opportunity exists for the transfer of genetic traits from a genetically modified organism to a wild (and potentially weedy) relative, a potential problem exists. The problem poses three relevant questions: (1) does the genetically modified crop have extant relatives, (2) what is the extent of hybridization between crop and relative in nature, and (3) what is the current ecological role of the relative in natural ecosystems?*

One of the main concerns in the introduction of transgenic plants is the potential for the development of a new weed or forms of an existing weed (Tiedje et al., 1989; and Keeler, 1989). Serious weediness is generally related to 10-12 traits (Keeler, 1989). Transgenic plants that are most likely to be introduced in the near future will involve one or a few changes for pest or stress resistance or processing qualities. This should mean that they will be easier to follow and less likely to result in weediness. There is evidence, however, that a change in a few characteristics can make a plant a weed, particularly where these changes involve pest resistance or fecundity in a new environment.

The introgression of transgenes into natural populations and their impact on the environment and on weediness of natural populations has been a concern. One does need to consider the possibility of gene transfer to relatives, particularly when compatible species exist in the areas where the transgenic plants are proposed to be grown. Dale (1992) has provided a partial list of crops that are being genetically modified and their sexually compatible relatives. This is a starting point. Ellstrand and Hoffman (1990) offer a more comprehensive discussion on this point. They note that many centers of crop diversity occur in tropical areas, so these would be areas where compatible relatives are likely to occur. They discuss a number of ways that risks could be reduced such as isolation by distance, cultural methods such as avoidance of the same flowering time and isolation by genetic methods such as the introduction of male sterility. So there are some things that can be done to reduce or retard gene introgression, even if we are introducing transgenic material into areas where compatible species exist.

The risk of horizontal gene transfer must also be considered. Horizontal gene transfer is when genes are transferred between plant species or between plant and non-plant species without sexual transmission. With naturally occurring vectors such as *Agrobacterium* and transposable elements and the degree of gene homology between different organisms, this seems like a genuine possibility. However, the likelihood of it being a significant factor appears negligible and thus the biohazard risk is viewed as negligible (Prins and Zadoks, 1994; and Schlüter et al., 1995).

Gene flow

Gene flow among species has been studied relative to the potential for transgenes to be transferred to wild relatives. In general it has been difficult to differentiate between gene flow due to hybridization and introgression, convergent evolution, or plasticity. Doebley (1990) did an extensive study of gene transfer from maize to the teosintes. Morphological and molecular data suggest that introgression goes both directions at low levels. Because of teosinte's limited distribution, the potential for transfer can be easily limited.

Gene flow between cultivated and free-living (wild) forms has been demonstrated for other crop plants. There is some evidence indicating a close relationship between crop/weed populations such that companion weeds are a part of a genetically interactive crop/weed population system in which coevolution occurs via hybridization/differentiation cycles (Wilson, 1990). In the case of sunflowers, hybridization between cultivated and wild species is known to occur as they coexist in sunflower fields. It was found that gene flow decreased with distance from 27% to 2% at 3 m and 1000 m from the source, respectively (Arias and Rieseberg, 1994). Physical distance did not prevent gene flow in the case of sunflowers. The authors suggest it may be necessary to use both physical distance and chromosomal incompatibility for an effective, though not impermeable, barrier to gene exchange. In studies of oilseed rape, it has been reported that there is no indication that kanamycin or herbicide tolerance increased the invasive potential of this crop and, if anything, transgenic lines were less invasive and less persistent than their natural counterparts (Crawley et al., 1993). Gene transfer has been documented for several crops such as the amaranths (Sauer, 1967; and Tucker and Sauer, 1958), from rye to its wild relatives (Jain, 1977; and Suneson et al., 1969), and African rice (Second, 1982). None of the hybrids were more aggressive, nor did they have an enhanced range, but they were more crop-like. Johnson grass hybrids on the other hand provide an example of the type of risk associated with gene flow from crops to weedy relatives (Warwick et al., 1984). One can conclude from these and other studies that hybridization can occur between cultivated and wild species for some crops and therefore transgenic gene flow will occur unless steps to prevent it using physical barriers, male sterility, and genetic modification are taken.

> **Discussion:**
>
> *Q: What should be the isolation distance between the transgenic potatoes and any cross breeding species?*
>
> R: The distances used in Northern countries are derived from the experimental work done on pollen transfer distances. For example, in a recent study it was shown that no transfer of pollen from transgenic potatoes was noted beyond 20 meters from the test plot.* In the United States an accepted isolation distance is one-quarter of a mile if there are no pollen dispersal controls in place. While agreeing that research performed in United States and Europe would give Latin American researchers/regulators useful hints, all results are not necessarily valid in Latin America. For example, effective isolation distances may be different in the region because of the difference in pollinators and intercrossable species. Consequently, additional Latin American surveys of cross-breeding varieties are needed to help with genetic analysis. Site specific studies were recommended and ecological impact research of certain traits in the region are also necessary.
>
> * McPartlan, H.C. and Dale, P.J. 1994. An Assessment of gene transfer by pollen from field grown transgenic potatoes to non-transgenic potatoes and related species. *Transgenic Res.* 3:216-225.

Kaptejins (1993) did case studies for the risk of transgene introduction for potato, beet, oilseed rape and maize for the Netherlands. Potato and maize are genetically isolated from wild species they might cross with there, but beet and oilseed rape have wild relatives in the Netherlands with which they could potentially hybridize. One can conclude from this study that the risk transgenic crops pose to the environment from gene transfer depends on the characteristic of the host species.

The potential for transgenes in potato to introgress into natural populations has been evaluated for the U.S. and Canada (Love, 1994). Love concludes that there is no threat because the species native to the southwestern United States are highly unlikely to hybridize with tetraploid cultivars and transgene introgression therefore is unlikely. In an evaluation of the potential for transgenic introgression into centers of diversity in Mexico and Costa Rica, species were identified which could hybridize with transgenic tetraploid cultivars. Therefore, precautions would need to be taken in areas where these species grow to avoid introgression of transgenes into native populations (Hanneman, 1993). Outcrossing rates have been shown to be significant in natural settings. Brown (1993) reported outcrossing rates of 0.10-1.0 for tetraploid cultivars of *S. tuberosum* ssp *andigena* and ssp *tuberosum* grown in Peru, while Rabinowitz etal. (1990) noted that 95% of the seed from a mixed planting in the Peruvian altiplano of the cultivated diploid *S. stenotomum* and the wild diploid species *S. sparsipilum* were of hybrid origin. Clearly natural hybridization must be considered.

Vayda and Belknap (1992) in a review of the potential for transgenic potatoes noted that there was no evidence of widespread dispersal of transgenic potato and that a six meter border could insure containment. Transgenic volunteers were detected in test plots, but these could be culled with standard practices and herbicide treatment. McPartlan and Dale (1994) found that gene transfer occurred at a rate of 24% in adjacent rows, at 2% at three meter and 0.02% at 10 m. At 20 m no transfer was noted. No gene transfer occurred between transgenic potatoes *Solanum dulcamara* and *S. nigrum*. The principal pollen vector in this study was presumed to be the bumble bee. In another study, Skogsmyr (1994) reported gene flow as high as 72% at 0-1 m, and ranged from 30-34% at 10 m, 23-36% at 100 m, and 20-31% at 1000 m. This rate seems extremely high compared to other reports, but the principle vector in this study was a small beetle capable of flying long distances. It is worth noting the difference in dispersal results between these two latter studies which is assumed to be due to the principle pollen vectors involved. Eijlander and Stiekema (1994) in studies with *S. nigrum* and *S. dulcamara* noted that the latter was incongruent with potato at all ploidy levels, but *S. nigrum* displayed unilateral incompatibility. *S. nigrum* had to be emasculated to form berries and promote seed development or its own pollen would out-compete potato pollen. Embryo rescue had to be used to obtain hybrids. It was concluded that gene flow between potato and its most common relatives in Western Europe is highly unlikely. Again it is evident that it is important to determine the vector(s) in an area where transgenes are to be introduced in order to design appropriate containment measures.

Potato: gene exchange in centers of diversity

Let us now turn our attention to potato and the introduction of transgenic potatoes into areas where related species are present. There are two principle centers of diversity for potato, one in Mexico and the other in Peru, Bolivia and Northwest Argentina. Related tuber-bearing species are found from southwestern United States to southern Chile and Argentina, largely following the mountain chain (Hawkes, 1990). More than 500 species have been described and they represent a polyploid series from diploids to hexaploids. Figures 1a and 1b indicate their distribution by series (Hawkes, 1990).

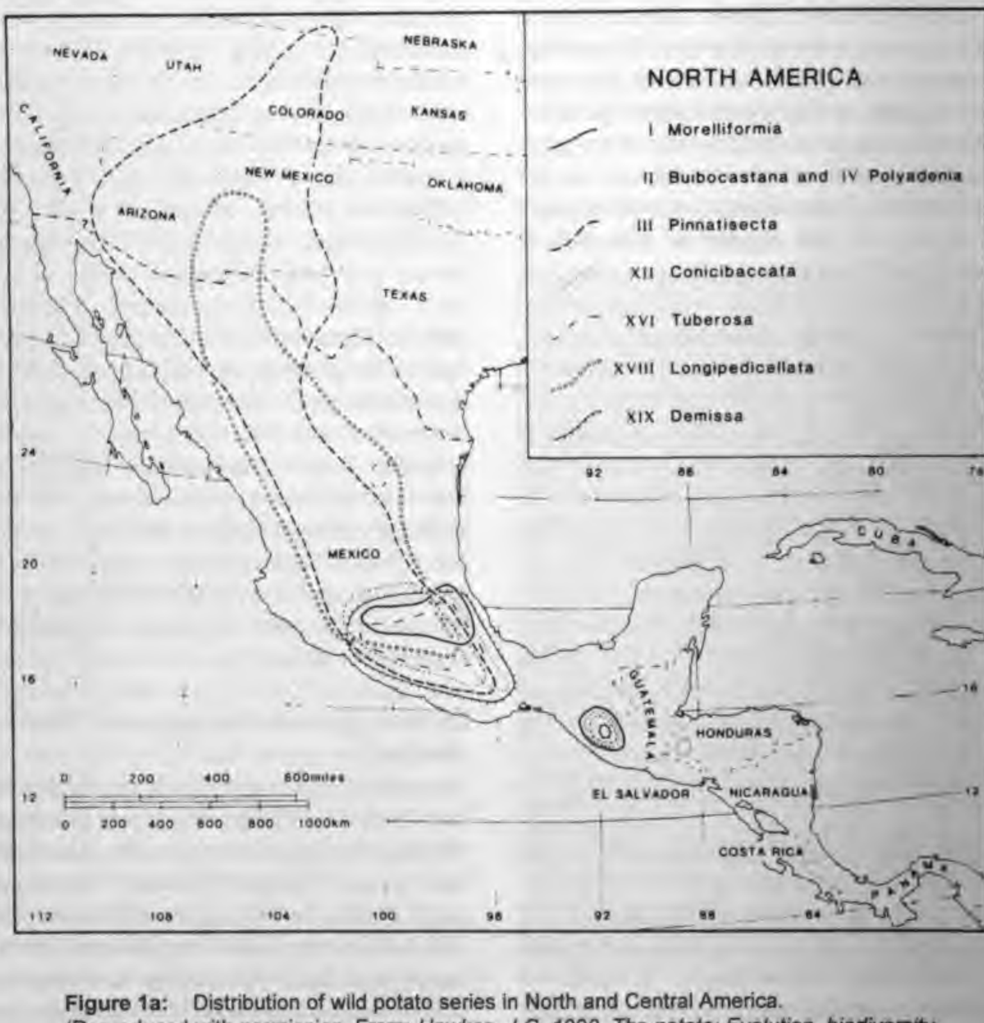

Figure 1a: Distribution of wild potato series in North and Central America. (Reproduced with permission. From: *Hawkes, J.G. 1990. The potato: Evolution, biodiversity and genetic resources.* Belhaven Press, a division of Pinter Publishers, 25 Floral St., London WC2E 9DS. All rights reserved.)

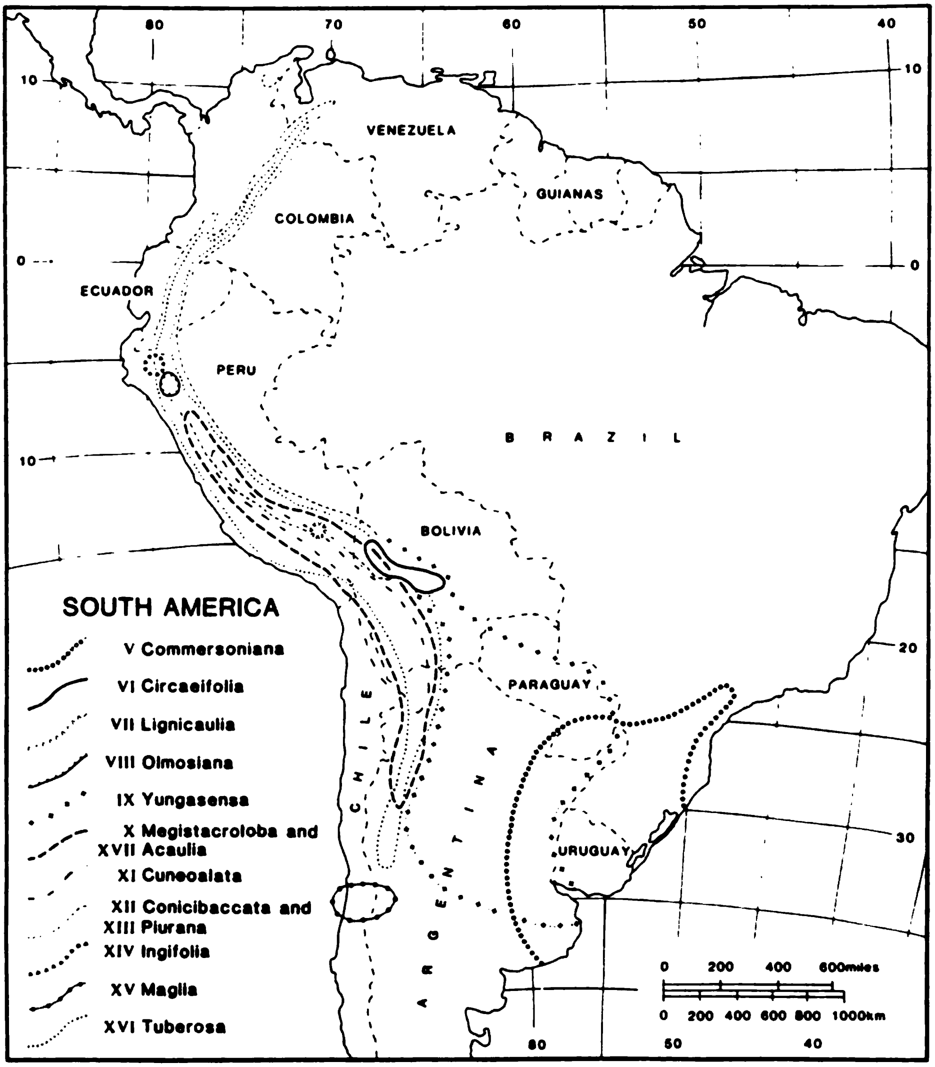

Figure 1b: Distribution of wild potato series in South America.
(Reproduced with permission. From *Hawkes, J.G. 1990. The potato: Evolution, biodiversity and genetic resources.* Belhaven Press, a division of Pinter Publishers, 25 Floral St., London WC2E 9DS. All rights reserved.)

Taxonomically the tuber-bearing species have been delineated by their morphology, ploidy and geographical location. They are in the Solanaceae family, the genus *Solanum L.*, the subgenus *Potatoe* (G. Don) D'Arcy, Section *Petota* Dumortier, Subsection *Potatoe* G. Don., which is further divided into series and finally species.

Discussion:

Q: Is there a need for more studies on gene flow in South America?

R: A lot of testing has been done in Northern Countries* and this experience may be useful for countries in South America. The use of information derived from work performed elsewhere should be done with specific reference to the test under question and the conditions of that test. One also has to question the reliability of the information and perhaps proceed on a case by case basis. It would be useful for Latin American regulators to have discussions with the experienced authorities from other countries who have considered isolation distances, agronomic practices, and other field trial details. Their experience can be incorporated in Latin American evaluations to provide assurance of biosafety. Evaluation procedures and monitoring measures that have been used to date have been accepted by regulators and can be done at low cost. Transgenic crops at the commercialization stage may present different concerns or different levels of concern. These have to be considered in more detail.

Q: Should it be assumed that there will be gene flow from transgenic crops to intercrossable wild species in Latin America?

R: Isolation may be effective at field trial stage, but no specific distance should be considered absolute. It could be assumed that, if transgenic potatoes are commercially released where interfertile species exist, there will be gene flow. When considering isolation, it is important not to generalize and consider all of South America as being essentially the same. There are wide variations in climate on a country to country basis and regional differences in agricultural practice (e.g., farm size, chemical use and crop rotation, etc.) that will have impacts on gene flow. There is a wide diversity in the abundance of wild *Solanum* species in different geographic areas (e.g. in two-thirds of Argentina there are no wild *Solanum* species).

*The United States was specifically mentioned, but work in Europe was also recognized.

To understand the potential for hybridizations one needs to consider 2n gametes, stylar barriers and endosperm balance numbers (EBN). Most species exhibit some level of production of 2n gametes (den Nijs and Peloquin, 1977; Novy and Hanneman, 1991; and Watanabe, 1988). The presence of 2n gametes offers the possibility of the species not only to hybridize with those of its own ploidy level but also with others of higher ploidy levels. This then provides a means for genetic exchange between members of different ploidy levels (den Nijs and Peloquin, 1977).

Stylar barriers are a factor in both intra and interspecific hybridization (Fritz and Hanneman, 1989). This interaction may be due to "S" alleles, incongruity, or undescribed interspecific barrier/isolating systems (Abdalla and Hermsen, 1972; Camadro and Peloquin, 1981; Dionne, 1961; Grun and Aubertin, 1966; Hermsen, 1978; and Pandy, 1962). These mechanisms of interspecific incompatibility generally lead to the stoppage of pollen tube growth in the upper portion of the style, preventing fertilization of the egg.

A developed endosperm is essential for nurturing the embryo and is another means of controlling seed development. The endosperm balance number hypothesis has been described in potato and states that the maternal and paternal EBNs must be in a 2:1 ratio in the endosperm for normal seed development to occur (Johnston et al., 1980). EBNs are independent of ploidy, but have been described as the "effective ploidy" of the parent. EBNs have been assigned to most species and can be used to group species into crossing groups - 2x(1EBN), 2x(2EBN), 4x(2EBN), 4x(4EBN), 6x(4EBN) (Hanneman, 1994; Hawkes and Jackson, 1992; Johnston and Hanneman, 1980, and Johnston and Hanneman, 1982). To simplify this concept, when the EBNs of the

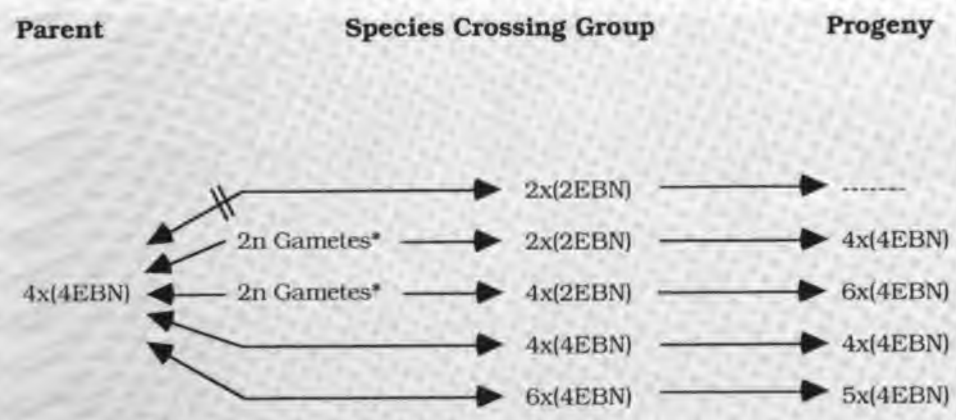

Figure 2: Potential crosses and progeny from hybridizations of 4x(4EBN) potato plants with various crossing groups based on 2n gametes and endosperm balance number (EBN). *Only possible with 2n Gametes from 2 EBN parent.

parents match, then those species are potential partners barring the occurrence of stylar barriers (Figure 2). This also means that, if 2n gametes are present, the species can also match with one of a higher ploidy level since EBNs are additive. For example, a 2x(2EBN) species can only cross with another species which has been designated as 2EBN [i.e. 2x(2EBN) or 4x(2EBN)], but with 2n gametes they can also behave as though they are 4EBN and thus cross with 4x(4EBN) or 6x(4EBN) species, thus widening the possibilities for gene transfer. An understanding of crossing relationships and the principles that govern them are essential to the discussion of the potential for introgression of transgenes into native wild species populations.

When one considers gene exchange in centers of diversity or where related species are common in the wild, field borders or the fields themselves, one is in a very different situation than in the United States or Canada. Here few species exist and then only in the southwestern part of the United States. They are essentially isolated from production areas geographically and by crossability barriers. The risk of escape of transgenes into native populations here is extremely unlikely (Love, 1994). What is the possibility for gene exchange where the transgenic cultivar is most likely to be 4x(4EBN), the ploidy and EBN of the common potato, *Solanum tuberosum*? We know that 4x(4EBN) potatoes can cross directly with other 4x(4EBN) species and also with 6x(4EBN) species. That means that they can cross with all of the species listed in Tables 1 and 2 and others that are not listed but are either 4x(4EBN) or 6x(4EBN). They can also cross with 4x(2EBN) (Table 3) and 2x(2EBN) (Table 4) species if they produce 2n eggs or 2n pollen. The frequency of 2n gametes is

TABLE 1: 4X(4EBN) WILD AND CULTIVATED SPECIES: HABITAT AND COUNTRY WHERE FOUND (Based on Hawkes, 1990)

Species	Country	Habitat
S. gourlayi	Argentina Bolivia	dry pre-puna to puna vegetation among grasses, cacti and small shrubs, 1900-3600 m
*S. oplocense**	Argentina Bolivia	dry thorn-bush scrub, hedges, cultivated fields and cactus vegetation, 2600-3500 m
*S. x sucrense**	Bolivia	weed of cultivation, among cacti and xerophilous shrubs, 2600-3900 m
S. tuberosum		
ssp *tuberosum**	Chile	cultivated, low altitudes, 500-2000 m
ssp *andigena**	Argentina Bolivia Colombia Ecuador Guatemala Mexico Peru Venezuela	cultivated, high altitudes, over 2000 m

*Grown in cultivated fields.

TABLE 2: 6x(4EBN) WILD SPECIES: HABITAT AND COUNTRY WHERE FOUND (Based on Hawkes, 1990)

Species	Country	Habitat
S. moscopanum	Colombia	humid pastures and waste places, 2900-3400 m
*S. oplocense**	Argentina Bolivia	dry thorn-bush scrub, hedges, cultivated fields and cactus vegetation 2600-3500 m
S. albicans	Ecuador, Peru	alpine meadows, 3000-4750 m
S. brachycarpum	Mexico	pine and *Abies* forests, 1700-3350 m
S. demissum	Mexico Guatemala	pine and *Abies* forests, in shade, 2650-3800 m
S. guerreroense	Mexico	pine-oak forests, 2800-3000 m
S. hougasii	Mexico	pine-*Abies* forests, 1600-2950 m
S. iopetalum	Mexico	pine-oak forests, east-facing escarpment slopes, 1900-2380 m
S. schenckii	Mexico	pine forest and among bushes, 2600-3000 m

*Grown in cultivated fields.

TABLE 3: 4X(2EBN) WILD SPECIES: HABITAT AND COUNTRY WHERE FOUND (Based on Hawkes, 1990)

Species	Country	Habitat
S. agrimonifolium	Guatemala Honduras Mexico	cloud forest, humid soils, 1600-3300m
S. oxycarpum	Mexico	humid pine forest and clearings
S. violaceimarmoratum	Bolivia	mountain rain forest, 3000-3600 m
S. acaule		
ssp *acaule*	Argentina Bolivia Peru	alpine meadows, paths, walls, ditches, arable fields, 3700-4200 m
ssp *aemulans*	Argentina	open stony places, alpine meadows, 2950-3500 m
ssp *punae*	Peru	alpine meadows, paths, walls, ditches, arable fields, 3700-4200 m
S. fendleri		
ssp *arizonicum*	Mexico USA	pine forest clearings, roadsides, 2000-2550 m
ssp *fendleri*	Mexico USA	dry oak-pine forests, not dense shade, 600-2800 m
S. hjertingii	Mexico	dry pinon scrub, 1750-2500 m
*S. matehualae**	Mexico	along field borders
S. papita	Mexico	open juniper, oak and pine woodland and scrub and among rocks and herbs, in rich soil, 2150-2800 m
*S. polytrichon**	Mexico	oak forests, Opuntia scrub, waste places and as field weed, 1800-2500 m
*S. stoloniferum**	Mexico	dry plateaus, valleys and hillsides

*Grown in cultivated fields.

TABLE 4: A PARTIAL LISTING OF A 2X(2EBN) WILD AND CULTIVATED SPECIES: HABITAT AND COUNTRY WHERE FOUND (Based on Hawkes, 1990)

Species	Country	Habitat
*S. bukasovii**	Peru	puna formation, roadsides, waste places, walls, rocky and shrubby areas, pastures, 3300-4300 m
*S. canasense**	Peru	rocky gravelly slopes, field borders and roadsides, 2900-4100 m
*S. marinasense**	Peru	rocky slopes among shrubs, in hedges, field borders, 2200-2700 m
*S. sparsipilum**	Bolivia Peru	fields, walls, field borders, 2400-3800 m
*S. huancabambense**	Peru	field borders, roadsides, 1800-3000 m
*S. chacoense**		
ssp *chacoense**	Argentina Bolivia Paraguay Uruguay	waysides, pastures, arable land, scrub and woodland margins, sea level-2350 m
ssp *muelleri**	Argentina Brazil	grasslands, river banks, waysides, fields, forest margins and clearings, to 800 m
*S. astleyi**	Bolivia	cultivated fields and waste places, 3000-3300 m
*S. boliviense**	Bolivia	dry scrub vegetation and cultivated fields, 2600-3750 m
*S. megistacrolobum**	Argentina Bolivia Peru	high mountain grasslands, field borders, 3500-4450 m
*S. toralapanum**	Argentina Bolivia	wetter habitats, field margins, rocky and grassy slopes, 3000-4100 m
*S. spegazzinii**	Argentina	dry interandine valleys, shade of trees and bushes, fields, field borders, 1900-3100 m
*S. ajanhuiri**	Bolivia Peru	cultivated, high altitudes, 3800-4100 m
*S. phureja**	Bolivia Colombia Ecuador Peru Venezuela	cultivated, wet mountain slopes, low altitudes
*S. stenotomum**	Bolivia Peru	cultivated, high altitudes

*Grown in cultivated fields.

TABLE 5 2x(1EBN) WILD SPECIES HABITAT AND COUNTRY WHERE FOUND (Based on Hawkes 1990)

Species	Country	Habitat
S. brevidens	Argentina Chile	
S. etuberosum	Chile	dry mountain forests, 1250-2500 m
S. fernandezianum	Islands of Juan Fernandez	wet forests, 100-600 m
S. bulbocastanum		
ssp *bulbocastanum**	Mexico	woods, grasslands, rocks and field borders
ssp *dolchicophyllum**	Mexico	
ssp *partitum**	Mexico Guatemala	
S. brachistotrichum	Mexico	dry pinion scrub vegetation, 1750-2500 m
S. cardiophyllum		
ssp *cardiophyllum**	Mexico	dry scrub vegetation, waste places, field borders, old lava fields, weed of cultivation,1600-2550 m
ssp *ehrenbergii**	Mexico	
S. hintonii	Mexico	stone walls, among low shrubs, under trees, 1700-1850 m
S. jamesii	Mexico USA	dry scrub vegetation
S. x michoacanum	Mexico	damp grassy fields, among rocks, 2000-2100 m
*S. nayaritense**	Mexico	maize fields, undisturbed natural vegetation
*S. pinnatisectum**	Mexico	cultivated fields, waste places, field borders
S. stenophyllidium	Mexico	dry hilly rangeland with small oaks, shrubs and Opuntias
S. tarnii	Mexico	open vegetation of small shrubs and herbs, among rocks with pines or oaks close, 2360-2650 m
*S. trifidum**	Mexico	oak and pine forests, partial shade, maize fields, roadsides, 2100-2400 m
S. commersonii		
ssp *commersonii**	Argentina Brazil Uruguay	coastal areas, marshy places, fields, river banks, woods and sandy shores, sea level-400 m
ssp *malmeanum**	Argentina Brazil Paraguay Uruguay	similar to above, more inland prefers shady thickets and woodlands, sea level-400 m
S. chancayense	Peru	coastal hills, 150-550 m
S. mochiquense	Peru	in mountains, 1650-1700 m, coastal hills, rocky hillsides, 250-500 m
S. lignicaule	Peru	dry brushy slopes, old cultivated terraces, 3000-3500 m
S. capsicibaccatum	Bolivia	cloud forest, among bushes, 2000-4000 m
S. circaeifolium	Bolivia	cloud forest, scrub vegetation, 2500-3600 m

*Grown in cultivated fields.

relatively low for the 4x(2EBN) species, so while hybrids are possible, the chances of them occurring are low. If they did occur, they would be 6x(4EBN) and would not be able to cross with 4x(2EBN) species because of EBN differences, unless 2n gametes functioned again. However, they could hybridize with 6x(4EBN) species if they were present in the plant community - a major concern. The products of the crosses with 2x(2EBN) species would result in 4x(4EBN) hybrids via 2n gametes, which could cross with other 4x(4EBN) species in their vicinity. Crosses between 6x(4EBN) species and 4x(4EBN) transgenic plants or species will result in 5x(4EBN) offspring which could cross to 6x(4EBN) species or to 4x(4EBN) species. A major stylar barrier is known to occur between 2x(1EBN) species and *Tuberosum* haploids when the latter are used as females (Novy and Hanneman, 1991). 2x(1EBN) species (Table 5) can not cross with 4x(4EBN) plants so they are not of concern. The real concerns then rest with 2x(2EBN), 4x(2EBN), 4x(4EBN), and 6x(4EBN) species where gene transfer can occur either directly or via 2n gametes. As has been mentioned, one also needs to consider not only the production of the hybrid but also its potential for further gene exchange. Because of the ploidy series and the prevalence of 2n gametes among the tuber-bearing Solanums, the situation of gene flow may not be as simple and straightforward as one might at first imagine. The nature of the pollinator is also extremely important and will influence gene flow among species and be a consideration in designing containment systems.

Where the introduction of foreign genes may be a threat to a native population(s), one must assess the environmental risk and decide if containment should be considered. If containment is necessary, what might be done? One could avoid areas where the threat is greatest; where 6x(4EBN) or 4x(4EBN) species are endemic and/or where native cultivated 4x(4EBN) forms are grown. As has been noted, the presence of pollinators is also a factor that must be considered. Bumble bees are generally regarded as the common pollinator of potato, because the flower needs to be vibrated for pollen collection. Bumble bees live in small groups. They typically will be found to nest in fields or the immediate surrounding area and generally forage close to their nests but may fly several kilometers if necessary (Heinrich, 1979). As was shown in the study by Skogsmyr (1994), there may be other effective pollinators, such as the small beetle in his plot, that may need to be considered. Armed with such knowledge, one may be able to set up safe distances from other cultivated or wild species in the area. The absence of effective pollinators would substantially reduce the chance of natural pollination. Biological barriers, such as male sterility, could also be used. If one chose a male sterile variety for transformation or introduced male sterility into the transformant of choice, this would significantly reduce the chance for gene exchange, even if it were female fertile, since bees tend not to visit male sterile flowers (Arndt et al., 1990; and Sanford and Hanneman, 1981). This is a "safe" solution even in areas where cross compatible species and varieties are present. Stylar barriers could also be used to reduce the chance of gene exchange with natural populations of wild species.

Gene exchange between transgenic 4x(4EBN) cultivated potato and its wild relatives is likely to occur given that it can cross with 2x(2EBN) and 4x(2EBN) species via 2n gametes, and directly with 4x(4EBN) and 6x(4EBN) species. To assess the situation in a given area one must know the species and the pollinators present in order to establish appropriate containment measures if deemed necessary.

The simplest means is to avoid these areas, select areas where pollinators are not present, or use male sterility. These precautions can greatly reduce the risk of introgression of transgenes into native populations.

> Discussion:
>
> *Q: Is it possible to regulate the use of transgenic potato differently in different regions and ban the use of certain varieties in centers of* Solanum *diversity?*
> R: Assignment of different geographical zones based on estimated risk for eroding *Solanum* diversity would be possible but of limited use. Transgenic crops/varieties will reach the critical regions sooner or later. In many cases there is a natural geographic exclusion of particular varieties of potato. For example, some varieties grown in Bolivia are not suitable for Argentina and vice versa. This may hinder the flow of genes between different regions.
>
> *Q: Does deregulation of a transgenic crop in, for example, the United States mean that the crop will reach the Andean region (even if it is not supposed to)?*
> R: There may be no effective way to prevent commercialized transgenic products from reaching a specific region.*
>
> * There were no extensive discussions on how phytosanitary rules (e.g. plant quarantine rules) could limit the unwanted movement of germplasm.

Decision making models

The work on the biosafety of kanamycin-resistant transgenic plants could serve as a good example of the thoroughness that might be necessary in the consideration of biosafety issues (Nap et al., 1992). In this review they note the length to which it was necessary to go in order to be sure of the effect of kanamycin resistance on the environment, and human and animal health: the known forms of resistance to the antibiotic, the potential spread through plasmids in bacterial populations, its potential effect on weediness, its addition to the soil, its spread through cross pollination, the potential for horizontal gene transfer, and its effect on human and animal consumption (Figure 3). The conclusion from all of these and other studies was that "kanamycin-resistant transgenic plants can generally be recognized as safe." It is a good model to be followed in cases of questionable impact on the environment. Another model that may be useful is that of the FLAVR SAVR tomato (Kramer and Redenbaugh, 1994).

The introduction of genetically modified organisms into the environment has raised both public and scientific concerns. To meet these concerns several methods have been proposed to aid in assessment of their safety. Kapteijns (1993) described a step by step method for assessment.

> *"The first step is to determine the level of safety concern of the unmodified organism. The following factors need to be taken into account: (1) the pest/pathogen status of the organism, (2) its ability to become established in the accessible environment, (3) its ecological interrelationships, function, and importance in the community, (4) its ability to transfer genetic information, (5) the potential for monitoring and control. The second step in the assessment would consider how the genetic modification affects safety. Three factors must be taken into account: (1) the process of genetic modification, (2) gene construction and expression, (3) the degree to which knowledge of the molecular biology and other information is available to predict the safety level of the modified organism relative to that of the unmodified organism. The third step would be to combine the evaluation in steps one and two."*

From this, the appropriate confinement can be determined such as physical, biological, chemical and/or environmental means.

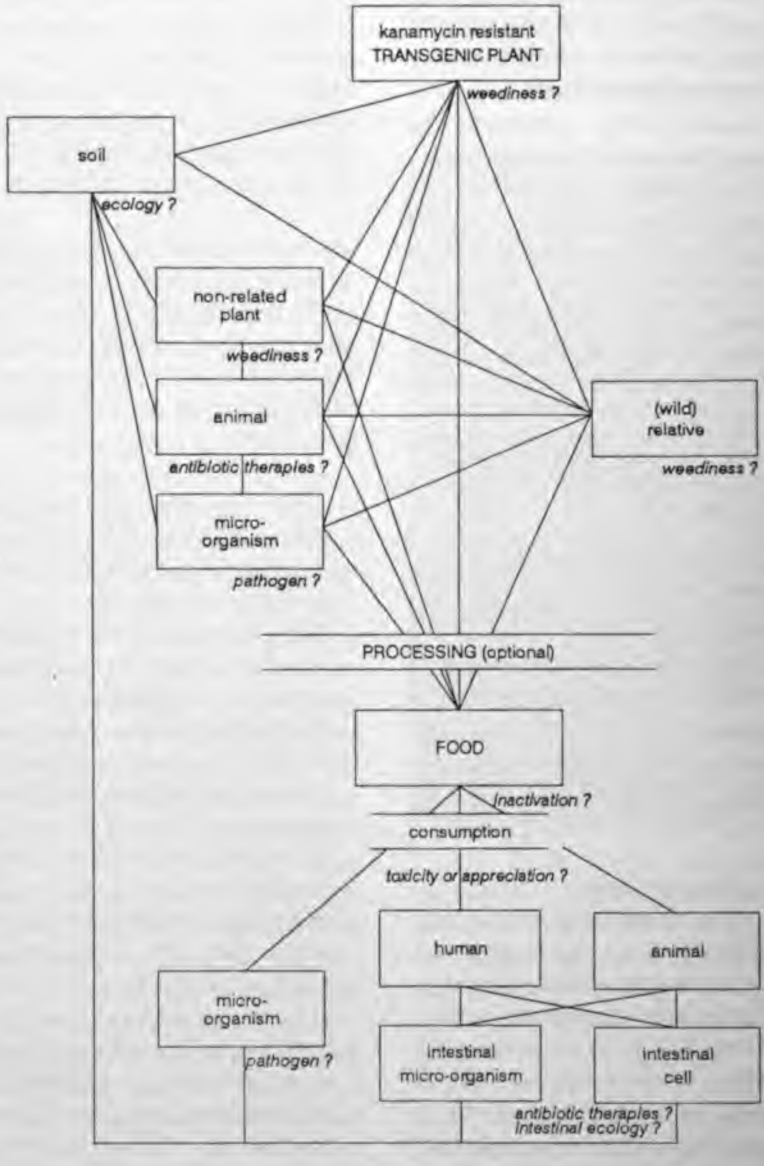

Figure 3. Potential effects of kanamycin resistance on the environment, and human and animal health. The questions below the boxes specify the nature of potential impacts. (Taken from Nap, J-P., Bijvoet, J. and Stiekema, W.J. 1992. Biosafety of kanamycin-resistant transgenic plants. *Transgenic Res. 1:239-249.* By permission of the authors and the publisher, Chapman & Hall, London).

The National Research Council report (NRC, 1989) provides a similar framework for decision making with regard to risk assessment. They deal with the questions of familiarity, confinement and environmental effects. In general, one can answer most of the questions dealing with familiarity quite readily, but questions concerning confinement and environmental effects are less definitive and the necessary data may not be available. This latter point is where we find ourselves in most issues dealing with hybridization and environmental risk associated with the introduction of transgenic plants.

Discussion:

Q: *Is the presence of "unneeded" genes significant?*
R: Marker genes or other "unneeded" genes are used in the development process and carry over into the final products. Recognizing that these are often unnecessary and maybe unwanted, there is now ongoing research on naturally occurring resistant varieties to see if their genes can be used instead of foreign tolerance genes. There is some question as to whether there is any benefit to this kind of research when there may be little real concern regarding the markers currently in use.

Conclusions

The level of risk a group is willing to accept has scientific, social and political determinants. Science can provide the basis and framework for decision making, and a logical decision-making scheme such as that proposed by the National Research Council can be followed (NRC, 1989). Overriding the scientific considerations will be local and national, social and political concerns (Siddhanti, 1991; and Tait, 1988). There may be resistance to the introduction or use of transgenics; there may be no market for it because of a reluctant population to use it; or there may be fears of how it may differ from a well known product. These perceived risks can be more important than the scientific determination and need to be understood and addressed if consensus is to be reached. The need for food, both for internal use and for export, may override the risks associated with the introduction of transgenics to the environment, especially where financial needs are great. There is also the matter of national pride that is associated with scientific accomplishment which also may drive this issue beyond the risks associated with it. These are not matters of irresponsibility, but rather deal with the practical decisions that must and will be made on a national basis.

The introduction of transgenics is no longer an issue of whether it will occur, but rather when and where it will occur. The fact that they will be grown in centers of diversity seems to be a certainty. There is a need to have dialogue to establish what position should be taken nationally and what degree of regulation should be established *vis-à-vis* the perceived importance of native species and the purity of their gene pools. These decisions will be made by each country. Centers of diversity are of value to worldwide agriculture, so the decisions made will also have global implications.

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Chapter 4
Environmental Concerns with Transgenic Plants/Potatoes - A General View

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Summary

This paper reviews the more common environmental concerns that have been raised following release of transgenic plants. It further specifically discusses the potential for adverse environmental effects following introduction of transgenic potatoes in Latin America. The authors note that research involving the use of genetically modified organisms is expanding in Latin American countries. As a consensus exists on the need to regulate the application of this technology, it is important to develop and harmonize biosafety regulations in Latin America. It is argued that international agricultural research centers should develop regulatory procedures that can be used or modified by national programs in developed and developing countries according to their needs and requirements. In this respect, the International Potato Center (CIP) has developed biosafety regulations that have been approved by the official authorities of the host countries.

Introduction

The human mind is always ready for change. After the discovery of restriction enzymes, the application of molecular biology techniques has allowed us to make new gene combinations in the laboratory, and transfer their sequences to a wide range of organisms (Dale, et al., 1993). In 1983 a gene coding for antibiotic resistance was introduced to the petunia plant (Bunders, 1990). Since then, a wide range of genetic modifications have been done in plants. They offer new opportunities for agricultural development because it is now possible to envisage a wide range of possibilities to reduce negative features or to increase the positive qualities of crops.

From 1987 to now the Animal and Plant Health Inspection Service (APHIS) has issued 689 permits to introduce genetically modified plants with herbicide tolerance, insect resistance, higher product quality, and viral and fungal resistance. Potato is the fourth most frequently used plant for transformation after corn, tomato, and soybean (NBIAP News Report on Internet in May 1995).

> **Discussion:**
>
> *Q: Are genetically modified plants different from traditionally bred plants?*
>
> R: This remains a controversial question. With indirect transformation experiments (e.g. somaclonal variation, protoplast fusion, etc.), it has not been necessary to take precautions and there are no special regulations in place. Although plants derived by such methods may be regulated elsewhere, there seemed general consensus that they were not necessary. When asked if scientists at the International Potato Center believe there is a difference between traditional breeding practice and recombinantly derived plants, the answer was yes.

At the International Potato Center (CIP), the production of transgenic crops (potato and sweetpotato) are considered a valuable complementary approach to classical varietal improvement through breeding. Pest and disease resistance are targeted by using antibacterial genes, anti-viral genes (coat protein constructs), and a *Bacillus thuringiensis* (*Bt*) gene for insect resistance (CIP, 1992).

Areas of concern

The application of genetic engineering for crop improvement followed by the release of transgenic plants in the environment raised many questions about the risks of these new artificial genetic combinations (Dale, 1992).

Concerns and questions often raised are:

1. The origin of the gene incorporated. One of the most common public myths is the thought that genes from related species are safer than from remote species or organisms. The origin of the gene is in fact of lesser importance than its function, product(s), integration site, and epistatic and pleiotropic effects (Hanneman, 1994).

2. The stability of the new genetic combination. Several studies in recent years seem to indicate that gene expression as well as its integration site are stably maintained across generations.

3. The advent of pollen released by transgenic plants. A common strategy is the manual removal of flower buds of transgenic plants to avoid pollen being spread in the environment. Transgenic potato fields should be surrounded by a minimal separation distance from wild relatives because cross pollination might occur. The dispersal of pollen carrying foreign genes is a principal risk for the environment associated with field trials of transgenic plants. So far, there have been many releases of genetically modified potatoes (Skogsmyr, 1994; and Tynan et al., 1990) and several experiments were performed to analyze pollen dispersion by bumble bees and wind in fields with both transgenic and non-transgenic plants, and to assess potential effects of cross pollination (McPartlan and Dale, 1994).

4. The impact on human health. Risks due to the manipulation or release of transgenic potato plants include possible adverse effects on

Discussion:

Q: Are there specific concerns regarding potential allergenic reactions from the ingestion of transgenic potatoes?

R: Development of allergenicity is a complex issue and dependent not only upon the genetic sequence but also the conditions under which the gene product is produced. It was suggested with respect to toxicity and allergy development, that the concerns with transgenic plants may be overstated *vis-à-vis* traditional breeding programs. The latter sometimes results in the production of compounds (such as terpenes, phenols and lectins) which independently or in combination can have negative effects on human health. In the case of transgenic plants, there is usually an extensive knowledge of the introduced gene product that includes testing for putative effects on human health. However, tests on gene products expressed outside plants are not enough. The nature of the protein, (e.g. glycosylation patterns and other modifications) often determines antigenicity and allergenicity and may be different in different organisms. Consequently, there is a need to look at health impacts when gene products are expressed in the host plants. This issue has been investigated at length in the United States but the results seem inconclusive. One reason for this is the inherent limitations with testing on a small-scale. Rare occurrence effects, such as allergenicity, may only be apparent following large-scale exposures, e.g. post commercialization.

Q: Northern companies have resisted the use of animal genes in their development programs because of potential public reaction. Does the same attitude prevail in Latin America?

R: There are no scientifically justified reasons for this. It appears more a matter of ethical considerations. There may not be the same level of concern in South American countries where the public acceptance of transgenic crops is higher. To countries facing urgent needs (e.g. food shortages) there is less resistance to transgenic crops with animal genes.

human health. Transgenic material should be tested in the laboratory to be sure that they represent no risk before their manipulation or consumption by humans (e.g. toxicity and allergy development).

5. The monitoring of transgenic plant introduction. A follow-up of the released transgenic potato plants is important to assess their possible negative effects on human health and the environment.

6. The modification of the natural ecosystem. Aside from cross pollination with wild species, transgenic potatoes may alter the ecosystem by modifying natural selection pressure on the fauna. The natural distribution of species, pathogenic or not, in the environment can be skewed toward a new equilibrium which may not be suitable for the preservation of genetic diversity. It is important to assess the behavior of genetically modified plants in new ecological niches in order to identify potential problems before they occur.

7. The type of agriculture involved. Large-scale (intensive) agriculture needs to follow national regulations for the use of transgenic potatoes. Sustainable agriculture will require provision of all necessary information and education to farmers.

Transgenic potatoes in Latin America

Genetic diversity studies of potato species have shown that the Andes in Latin America is the center of origin of potato. There are 235 potato species registered, from which only eight are cultivated (Huamán, 1983). Nearly all of these were originally tetraploid or were obtained via hybridization between two or more diploid species. Controlled and natural hybridization has created new genotypes that are suitable as recipients of improved traits. No special care was taken before release into the environment. In comparison, transgene flow should be considered specifically on a case by case basis. The concern that transgene flow into crossable wild species will induce weedy behavior in the new genetic combination lacks evidence. However, the issue can and should be addressed in the laboratory before releasing transgenic potatoes into the environment when crossable species are known to be present. There is little or no evidence of sexual compatibility with *Solanum* species living in temperate regions (Dale et al., 1993).

Risk assessment

Risk assessment should start in the laboratory. The weedy behavior and nutritive quality of transgenic potato can be assessed before release into the environment. Key experiments should be performed on a case by case basis. Obviously, viral protection through the coat protein mediated resistance and new starch composition of transgenic potatoes require different experimental protocols. Biosafety regulations should provide a legal enforcement means to prevent possible scientific misconduct. As laboratory tests are successful, transgenic potatoes should be tested in greenhouse conditions where it is easy to evaluate all features during plant development. Eventually, field release should not present any risk for human health or the environment. Pollen spread should be monitored at release sites where wild species are present. In such cases, male sterile genotypes should be preferably used as transgenic material.

Biosafety regulations at CIP

Weedy behavior of transgenic potatoes has not been observed. It seems unlikely that transgenic potatoes released in the environment will become a weed. Gene flow of transgenes to wild species can take place under specific circumstances and should be avoided. The resistance of transgenic potatoes against pests and pathogens could facilitate the emergence of new races potentially more virulent for other plants. However, this risk is not restricted to transformed plants and is commonly accepted in agriculture using new varieties with higher pest and disease resistance.

CIP is applying transgenic research to improve important commercial potato varieties for resistance against pests and diseases. The first field trials were made in Bolivia to assess frost tolerance of transgenic potatoes (Devaux et al., 1994). Another recent example began with transformation for insect toxins originating from *Bacillus thuringiensis* under a collaborative project with Plant Genetic Systems, Belgium. The evaluation of transgenic plants was first carried out under controlled conditions in incubation rooms and greenhouses, which gave valuable information on the expression of the introduced gene. However, an overall assessment of plant phenotype and performance can only be made by testing transgenic potatoes in a field environment. Fourteen transgenic lines of potato from Lt-8 and *Sangema* clones expressing the *Bt* toxin gene were tested in fields at CIP's San Ramon station to evaluate yields and resistance to potato tuber moth (PTM) under natural conditions. This experiment was carried out in accordance with CIP biosafety regulations which established internal guidelines for the safe application of biotechnology. These guidelines have been adopted by Peruvian authorities as their biosafety regulations for the introduction of genetically modified organisms in the environment. In this field study, four corn barriers surrounded the field in order to deter pollen spreading. Flower buds were manually removed and autoclaved although the genotypes were of low fertility in this environment. The risk of cross pollination with wild species was non-existent as they are not found in this particular environment.

Conclusions

The contribution to agriculture of genetically transformed plants will be important as two-thirds of the Third World's population depend on agriculture for its livelihood (Broerse and Bunders, 1991). Yield increase and reduced pesticide use will be the most significant advantage of transgenic crops for developing country agriculture. Recent advances in potato improvement have encouraged Latin American countries to increase their potato production for the rapidly expanding fast food industry (CIP, 1992).

Research involving the use of genetically modified organisms is expanding in Latin American countries. As a consensus exists on the need to regulate the application of this technology, it is important to develop and harmonize biosafety regulations in Latin America (Visser, 1994). International agricultural research centers should develop regulatory procedures that can be used or modified by national programs in developed and developing countries according to their needs and requirements. In this respect, CIP has developed biosafety regulations that have been approved by the authorities of the host countries.

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Chapter 5
Potential Environmental Concerns *vis-à-vis* the Introduction of a Specific Trait: Experiences with Potatoes Engineered to Express Insect or Virus Resistance

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Summary

The paper addresses environmental concerns raised for transgenic potatoes with increased resistance to insects or viruses and discusses whether transgenic potato cultivars are different from traditionally bred cultivars. If transgenic potatoes are cultivated in regions with interfertile species, gene transfer to wild relatives will occur. This has implications on the potential for increased fitness of wild relatives due to the introgression of transgenes coding for insect and virus-resistance. An examination of the risks in relation to the benefits of these improved crops is called for. Considerations need to include the possibility of gene escape, the ecological effects of this gene escape, and the consequence of transferred genes in the cultivar itself. Scientists can draw upon the extensive experiences from traditional breeding to establish a baseline prediction of the behavior of a transgenic potato. It is important that Latin America develop a uniform set of biosafety regulations for the development, transfer and field testing of transgenic plants.

Introduction

The following assessment of concerns regarding the release of transgenic potato plants in Latin America is written from the perspective of researchers responsible for the potato breeding and genetics program at Michigan State University. The program's goal is to develop new cultivars that are improved for pest resistance, agronomic performance, and quality traits. Our philosophy is to explore ways in which we can integrate the new technologies with a conventional breeding program to more effectively achieve our goal of improved cultivars.

One aspect of this program is the development and use of transgenic potato plants. We have brought together a team of scientists through the USAID-funded Agricultural Biotechnology for Sustainable Productivity (ABSP) project that is focused upon developing transgenic potatoes to incorporate resistance genes having a positive impact on potato production in Egypt and Indonesia. Traits we are currently introducing into potato are resistance to potato tuber moth (*Phthorimaea operculella* Zeller) and Potato Virus Y (PVY). Other traits our program is working with include resistance to Colorado potato beetle (*Leptinotarsa decemlineata* Say), Potato Leaf Roll Virus (PLRV) and fusarium dry rot along with starch accumulation.[1]

[1] The cooperating scientists of this research team are entomologists, Walter Pett and Ed Grafius, and plant pathologist, Ray Hammerschmidt.

Why are we using plant transformation techniques?

We see genetic engineering as a new tool for the plant breeder. Prior to this technology, plant breeding, in general, had limitations to gene transfer. Sexual compatibility and the fertility of resulting hybrids defined the boundaries of gene transfer. Genetic engineering techniques break down these barriers and offer the plant breeder the ability to introduce genes from non-traditional organisms (i.e., organisms outside the gene pool). The concept of genetic engineering, when considered in the plant breeding continuum, therefore, now changes our concept of how to introduce genes into the cultivated germplasm.

We classify the technique of genetic engineering as a new means of germplasm enhancement. The obvious advantage of this technique is that a specific gene can be introduced to a plant. Undesirable traits that may be associated with other genes from the donor organism are not part of the gene transfer process. The technology associated with genetic engineering is still in its infancy and we expect to see a greater array of genes available for plant improvement along with improved techniques to transform plants in the near future.

Our program is involved in research to apply genetic engineering techniques to the cultivated potato because this technology offers availability to new genes for genetically improving our cultivated germplasm. Following the introduction of a gene into a potato line, we can then use conventional breeding techniques for further variety development.

Perceived concerns

What are the perceived concerns about the utilization of transgenic potatoes? This ability to transfer genes between sexually incompatible organisms raises concerns and may present risks. Could these newly integrated genes in a crop pose ecological risks that outweigh their agronomic benefit? Concerns center around three general risks: 1) the transgenic crop plant will become a weed in the agricultural system or invade natural habitats, 2) the flow of engineered genes to wild relatives will lead to hybrid offspring that will become more weedy, and 3) the transgenic plant itself will be toxic or allergenic to humans, domestic animals, or beneficial wild organisms (Crawley et al., 1993).

Should we use transgenic crops?

Transgenic crops offer us potential agricultural benefits. There is great excitement generated by the promise of this technology. In contrast, environmental groups have raised concerns about the widespread adoption of transgenic crops (Rissler and Mellon, 1993). As scientists engaged in plant improvement, we look at the benefits and risks of using transgenic plants on a case by case basis. The process by which we weigh the risks and benefits involves examination of the crop, the transgene and its location, and their interaction through the context of the concerns outlined by Crawley et al. (1993).

We closely examine whether these risks apply to our research. At Michigan State University the primary focus of our transgenic research is the introduction of genes that confer resistance to insects and viruses. In the following, we provide our philosophies, experiences, and insights into using these genes to engineer the potato for insect and virus resistance.

Case study analysis

Is the release of transgenic potato cultivars different from that of traditionally bred cultivars? Huttner (1993) suggests that the first assumption for risk assessment should be whether the transgenic crops are fundamentally different from those bred conventionally. Plant breeders may argue that the process of plant transformation is not very different from plant breeding. However, a major point which should be considered is that the gene pool in which we can introduce genes has expanded. Genes that were not accessible conventionally can be transferred. There is the possibility of gene transfer to wild relatives. Since transgenes may be unique, we should take the time to examine the consequences of the release of transgenic potatoes. However, when we look at the safety assessment of transgenic potatoes, the emphasis should not be whether or not these plants possess any environmental or food safety risks but rather do these transgenic potatoes pose any new risks over the release of traditionally bred potato cultivars (Conner, 1994).

At Michigan State University our objective is to develop and test transgenic potatoes with *Bacillus thuringiensis (Bt)* toxin genes for insect resistance and viral coat proteins for virus resistance. With the *Bt* toxin genes we are developing potato plants resistant to the Colorado Potato Beetle (CPB) in Michigan and resistant to the Potato Tuber Moth (PTM) in Egypt and Indonesia. The promise with host plant resistance is to reduce insect damage and also reduce pesticide usage on potato. Less pesticide usage would benefit the farm environment through reduced exposure. We are using capsid genes to confer resistance to the two viruses with worldwide importance, PVY and PLRV. The incorporation of resistance to these two aphid-vectored viruses would enhance seed production systems in potato. Reduced virus titer in seed potatoes would improve the crop's productivity (Banttari et al., 1993).

Conventional potato improvement

What risks have conventional breeding techniques imposed? In North America, the improvement of the cultivated potato (*Solanum tuberosum* ssp. *tuberosum*) has over a century-long history. The process of conventional potato breeding involves hybridization between two elite breeding lines to combine desirable traits from each individual. Superior genotypes are maintained through asexual propagation. Because of the heterozygous nature of the crop, any sexual crosses will result in the loss of integrity of the desired genotype. Potato cultivars have been improved for quality and pest resistances through traditional breeding. There is also an established history of utilization of South and Central American *Solanum* species to enhance the cultivated germplasm. Potato breeders throughout the world have exploited these species to introgress resistance to diseases and insects and to improve other agronomic and quality factors. Today these improved cultivars are being grown in most countries throughout the world under various climatic conditions and these cultivars have had the opportunity to hybridize with the sexually-compatible wild and cultivated species. With numerous examples of gene flow between the cultivated potato and its related species in South America (Hawkes, 1990), are there examples of increased fitness of any species due to gene movement from improved varieties? From our literature search, the release of these improved cultivars has not led to any published reports of negative ecological consequences.

We can also ask whether the cultivated crop itself has become weedy through this process. Potential weediness of potato can be from two sources: volunteers not removed from the previous year's harvest (Lutman, 1977) or true potato seed (Lawson, 1983). In the United States the rotations employed in potato production have not promoted natural populations of cultivated lines outside the agricultural system. The worldwide distribution of the cultivated potato should provide a context in which we can examine the question of weediness. In addition, the issue of weediness of transgenic plants can be addressed in small-scale trials through careful post-trial monitoring (Conner, 1994). Our suggestion is that in Latin America, where different climatic conditions exist, if transgenic potatoes are to be released, the issue of weediness must be addressed.

Has traditional breeding led to any undesirable products in the potato tuber? Numerous *Solanum* species are very bitter in taste due to presence of potentially toxic amounts of glycoalkaloids. In modern breeding programs potato tubers of advanced breeding lines are routinely evaluated for their level of glycoalkaloids. In the 1970s, the cultivar "Lenape" was released in the United States and was discovered to have total glycoalkaloid levels several times higher than normal levels. This led to the withdrawal of this cultivar from the market (IFBC, 1990).

We recommend that, for risk assessment, we draw upon these traditional breeding experiences as a reference to establish a level of acceptable risk for the deployment of transgenic potatoes.

Gene transfer between transgenic potato cultivars and related species

What is the likelihood that we will have gene transfer between transgenic potato cultivars and related species? Let us now consider the aspect of location in our transgenic plant deployment. In the United States, there are no species interfertile with our cultivated potato. Therefore, we are not concerned about gene movement to *Solanum* species (Love, 1994). The same situation exists for Europe (Dale, 1992), Egypt, and Indonesia. Therefore, the risk of gene transfer from the deployment of transgenic potatoes is non-existent in these three locations.

In Latin America, the situation is different. In the center of origin and diversity for potato, intercrossing between cultivated and wild species has and does occur (Rabinowitz et al., 1990). Therefore, we can conclude that if a transgenic potato is commercially released where interfertile species exist, gene flow of transgenes to the wild populations will occur.

To assess the risk, we need to quantify the gene flow of transgenes in Latin America. Kareiva et al. (1991) dissect the problem of gene escape and gene spread into four components:

- the distance transgenic pollen is likely to travel from source plants,
- the frequency that transgenic pollen produces viable hybrid offspring, assuming pollen transfer,
- the relative fitness of the transgenic offspring, and
- the spatial spread of the gene in a weed population.

Standard field experiments to quantify gene flow should have well-defined sources of pollen, genetic markers that identify the pollen source, and several seed sampling sites surrounding the source.

A body of information exists regarding gene flow between related *Solanum* species. If one is considering the commercial release of transgenic potatoes in Latin America, the availability of related interfertile species in the locality of release needs to be assessed and the necessity for gene flow studies needs to be considered. Furthermore, if gene flow studies are conducted, then the information should be quantified so it can be factored into the risk assessment.

What is the fitness of the gene in wild populations?

If there are interfertile species in the same locality of the transgenic potato we can expect escape of the transgene from the crop. Therefore we must next address the fitness of the *Bt* toxin gene and the viral coat protein genes.

The *Bt* toxin gene, under high expression levels in the plant, should reduce feeding damage from the target insect. Under high pressures from either the PTM or CPB this resistance gene should have positive economic consequences upon the harvested crop. Does the *Bt* toxin gene have fitness outside the cultivated species? South America is not within the geographic range of the CPB (Weber and Ferrom, 1994) and will not be considered. The PTM is the most important pest of the potato in tropical and subtropical areas. In addition, severe infestations have been reported in the highland regions of South America (Van Rie et al., 1994). What would be the fitness of the *Bt* toxin gene in hybrids between the cultivated and wild species expressing the *Bt* toxin gene? At this time we are unaware of any published results that answer this question. However, we know that a number of wild *Solanum* species are noted for their various resistances to pests, diseases and viruses (Hawkes, 1990). Will the introgression of the *Bt* toxin gene specific to *Lepidopteran* insects lead to greater fitness in natural populations? Considering the diversity of biotic and abiotic stresses placed upon the *Solanum* populations, we feel that the *Bt* toxin gene would likely confer little, if any, fitness upon the genotype under most natural conditions. If we were planning to commercially release *Bt*-transgenic potatoes in South America, we would design experiments to quantify the fitness of natural populations containing the *Bt* toxin genes.

Virus resistance has been an objective of numerous potato breeding efforts during the past 50 years. The advantage of virus resistance in the cultivated potato is found in seed production. Numerous *Solanum* species have been identified to have resistance to the major viruses and have been utilized to transfer these resistances to cultivated germplasm (Ross, 1986). Hence, the deployment of virus resistant cultivars has occurred throughout the world with non-significant risk. Would the escape of viral coat protein genes in Latin America increase fitness of hybrids between the cultivated and wild *Solanum* species? Drawing upon traditional breeding experience, the escape of virus resistance from cultivated to wild species would not likely lead to any selective advantages over time.

DeZoeten (1991) addresses a concern of using capsid genes to engineer resistance to viruses. Can the wide deployment of potatoes transgenic for coat protein genes lead to viruses with new host ranges? The examination of transcapsidation should be part of the risk assessment. Greene and Allison (1994) have demonstrated that recombination can occur between the mRNA transcript of the coat protein and the infecting virus. The results of these types of experiments can be used to establish the risk of commercial deployment of viral coat protein genes in potato.

A common food safety issue that comes to mind is whether the *Bt* toxin gene poses any health risks in the edible parts of the crop? The International Food Biotechnology Council (1990) thoroughly investigated the perceived hazards related to food safety of transgenic organisms. These concerns were for: protein products of the transgenes, pleiotropic and secondary effects resulting from expression of the transgenes, and insertional mutagenesis as a consequence of transgene integration. They concluded that genetic engineering does not pose any new food safety risks over those associated with traditional plant breeding. However, to ensure public acceptance of transgenic plants, it was recommended that the regulatory agencies proceed with caution until more experience with foods from transgenic plants is acquired. Monsanto has conducted an extensive food and feed safety assessment of *Bt*-transgenic cotton. Their experiments have shown that the introduced proteins from *Bt* var. *kurstaki* and neomycin phosphotransferase are safe (Fuchs, 1994). Moreover, this transgenic cotton was shown to be substantially equivalent to a commercial cotton variety in nutritional quality. Monro et al. (1993) found no nutritional or toxicological difference between a potato cultivar and its transgenic-derived potato for herbicide resistance. Conner (1994) recommends that transgenic potatoes be examined for glycoalkaloid concentrations as is routinely done in potato breeding programs.

Discussion:

Q: How effective would the Bt toxin containing plants have to be (i.e. plant pest tolerance level) in order to avoid resistance development?

R: It has been estimated by some that 400 times the level will be necessary. United States agencies (FDA, EPA, USDA) are looking at development of insect resistant management issues. It is not in the interest of the industry/breeders for insects to develop resistance to *Bt-transgenic* plants. Nevertheless, it was agreed that resistance for specific strains may occur and for some, it is expected to occur. Conventional and routine agricultural practices may be used to delay development of resistance. One view was that market forces should provide a sufficient incentive to avoid losing a competitive transgenic plant line. In the United States, management techniques aimed to delay resistance will take a concerted effort. The Government is not expected to play a significant role so the burden would fall on the industry. (Monsanto Company has initiated resistance management strategies recommended for its transgenic potatoes expressing *Bt* toxin.) In centers of diversity, where the wild relatives could act as refugia, the development of resistance may be retarded. Hence, in centers of diversity there are probably less problems with development of resistance. It was agreed, however, that the process of development, evaluation, and implementation of resistance management options would be a significant aspect of commercializing potatoes expressing *Bt* toxin.

In Michigan, like other potato growing regions, we are concerned with the consequences of the deployment of *Bt*-transgenic potatoes. Our concern focuses upon the management of the *Bt*-based resistance. The high expression of the *Bt* toxin gene in the plant imposes a very strong selection pressure upon the CPB for resistance to this toxin. The loss of refugia for CPB due to the extensive deployment of *Bt*-transgenics could quickly reduce the effectiveness of this host plant resistance. We are focusing research efforts to develop strategies in which to make the deployment of *Bt*-transgenic potatoes more sustainable. The sustainability of the *Bt*-host plant resistance should be addressed for Latin America.

Conclusions

The objective of potato breeders is to develop improved cultivars. As we identify superior genotypes we follow avenues to commercialization. We are using transformation as a means to introduce genes to enhance our cultivated germplasm. Being at the applied end of the development of transgenic plants, we focus our efforts on genes which have agronomic value (*Bt* toxin, viral coat protein and starch modification genes). We consider these genes to have a comfortable level of familiarity due to extensive research experiences. In the United States, these genes in potato pose little risk to the environment and have negligible food safety consequences.

We feel that it is important that Latin America develop a uniform set of biosafety regulations for the development, transfer and field testing of transgenic plants. If we were to commercialize transgenic potato cultivars with insect and virus resistance for Latin America, we would examine the risks of using these plants in relation to the benefits of improved crops. What do we define as risks? These include the possibility of gene escape, the ecological effects of this gene escape, and the consequence of these new genes in the cultivar itself. To establish a baseline prediction of the behavior of a transgenic potato, we would draw upon the extensive experiences from traditional breeding.

We feel that the risks of transgenic plants vary with the plant of interest, the engineered gene and location of the release. As familiarity with the engineered gene is gained and it is deemed benign, standard breeding practices to eliminate unwanted genotypes could be applied.

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Chapter 6
Searching for a Balance: Summary of Group Discussions

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Introduction

Having listened to general presentations and discussions on the environmental concerns for transgenic potatoes in South America, the workshop participants were divided into three groups to examine the issues in greater detail. Each group considered the more important transgenic traits currently being researched in their country and then articulated concerns *vis-à-vis* the stage of crop development (field trial or commercialization) and the anticipated benefits of improved varieties. The results of the work groups were reported at a plenary session. This chapter summarizes the thoughts and ideas expressed.

There was a clear consensus that the major efforts in transgenic potato development are, and will be, made in the evolution of plants with increased resistance to diseases and pests. The traits most analyzed at the workshop were resistance to insects, bacteria, viruses, fungi and abiotic stress (in particular to frost). Herbicide resistance and quality modifications (altered starch composition and protein content) were only briefly considered.

In the United States and Europe, the possibility of gene transfer to interfertile species is virtually non-existent due to the lack of wild relatives. In Latin American centers of origin there will be gene flow from commercially grown fertile transgenic potatoes to the wild *Solanum* populations. Therefore, the discussion was not so much concerned with the containment of transgenes as with the possible impact these transgenes would have on the environment; more specifically, the consequences of transgene flow in centers of diversity.

Environmental concerns and potential benefits

There was general agreement that the centers of diversity are of great value, not only locally but also worldwide. Furthermore, participants felt that one should take all reasonable measures to preserve this diversity. However, there was no clear consensus among the participants whether introduction of transgenic traits in wild and cultivated potatoes would erode *Solanum* diversity by changing growth patterns and thus displacing the natural population. Many felt that in most cases transgenic potato varieties would be no different than plants used in conventional breeding. Others argued that a trait which drastically increased the fitness (weediness) of plants would, at least theoretically, pose a threat to *Solanum* diversity. In general, there was a feeling that the available information and current research efforts in this field, specifically in relation to the Latin American situation, are inadequate.

Regarding the disease resistance traits (insect, bacterial, virus and fungal resistance) most participants took the view that the benefits would, in most cases, outweigh possible environmental impacts. It was argued that these traits were already present in the wild populations in many Latin American regions and that these traits are not different from those already selected for by potato breeders for many years. Therefore, the risks are not different from those associated with introduction of traditionally bred cultivars. Mention was made of the difficulty in comparing gene flow from transgenic crops resistant to, for example fungi, with traditionally bred or naturally occurring varieties. The reason for this is that a specific phenotype in the latter category is due to multigenic traits in contrast to transgenic plants where the same phenotype would most often be due to one single dominant gene. It was advocated that characteristics which are conferred by multigenic traits are much less efficiently transferred in comparison to those determined by a single dominant gene.

Summarizing the thoughts trait by trait (see Table 1):

Virus resistance

Virus resistance was thought to improve the sanitary conditions for growers and seed producers. Small-scale farmers would be most benefited since they, in many cases, do not have easy access to certified seeds. The genes encoding for virus resistance are already present in wild species and there is long experience with virus resistant potatoes obtained through traditional breeding. There was a limited discussion on the possibility of creating new viruses through recombination and transencapsidation in virus-resistant transgenic plants. It was recognized that some research has been done to address this possibility. Most of the participants thought this would not be a major problem considering that virus recombination through similar mechanisms already frequently occurs in nature. Therefore, no additional precautions in comparison to neutral genes were thought necessary.

Bacterial resistance

Transgenic plants coding for bacterial resistance were also seen as beneficial for the same reasons as in the case for virus resistance (see above). Regarding environmental concerns, it was pointed out that there are already genes encoding bacterial resistance in the wild plant population. In addition, it was mentioned that despite bacterial infections, plants are still able to flower and spread seeds. The infection affects tuber quality but not reproduction. Therefore bacterial resistance was not considered to be a trait that would dramatically increase the fitness (weediness) of potato plants.

Insect resistance

Insect resistance is also a trait relevant to most Latin American countries. The ratio of benefits versus potential environmental impact was high due to the negative effects of insecticides on the environment and human health. Economically, large-scale farmers who today are spending large amounts of money on insecticides would probably benefit more than small-scale farmers. Some argued that insect pests are not the major biotic stress on *Solanum* populations and therefore the presence of resistance genes would not increase the fitness of its host dramatically. As regards disruptive ecological effects (e.g. impact on "non-target" species), it was mentioned that most insects have alternative hosts which decrease the potential for development of insect resistance to gene products (e.g. *Bt* toxin). However, many felt that there was little available information on the impact of introgression of transgenes conferring insect resistance in centers of *Solanum* diversity. It was suggested that this trait would be suitable for a study on the effect of gene flow on selective advantage and disruptive ecological effects. In this regard a study was suggested of the possible disruptive ecological effects in different ecosystems (Andean region, tropical and subtropical) following a spray of *Bacillus thuringiensis*.

Fungal resistance

Of the fungal resistance traits, resistance to late blight (*Phytoptera infestans*) was most discussed. Participants agreed that transgenic potato plants resistant to late blight would be highly beneficial for Latin America, both in terms of increased productivity and a decreased need for fungicides. Both large and small-scale farmers would benefit. Considering environmental impacts, it was mentioned that there are species in the wild *Solanum* gene pool already resistant to late blight, therefore transfer of resistance genes would not dramatically affect the wild populations. However, some participants argued that the resistance to late blight may lead to increased fitness and that gene flow of this trait to wild relatives could change their growing pattern from non-weedy to weedy, possibly affecting *Solanum* diversity. Conversely, it was argued that wild *Solanum* populations were not under high selective pressure by late blight, as compared to cultivated varieties. This was due to unfavourable growing conditions for the late blight (wrong temperature, humidity, etc.) or due to natural resistance in the wild *Solanum* species. Consequently, it was argued that transfer of genes conferring late blight resistance would not have a major impact on the growing pattern of wild *Solanum* populations. The Toluca Valley, Mexico, an area with high *Solanum* diversity and late blight pressure, would make a good research site to explore the issue. The population there includes *S. demissium* species naturally resistant to late blight and a study of the impact of their competitive advantage on diversity was advocated.

Stress resistance

Frost tolerance was much discussed as an example of stress resistance traits. It was agreed that frost resistant plants would be beneficial in Andean regions and in the southern part of Argentina, increasing productivity and improving tuber quality. Naturally occurring frost resistance is encoded by multigenic traits. It was argued that this is an example where transfer of natural tolerance is more difficult than transfer of a single dominant gene encoding for a single protein conferring frost tolerance. When discussing environmental concerns it was argued that a frost tolerant trait, if spread to wild relatives, could possibly enhance fitness and subsequently weediness. However, frost tolerant transgenic varieties would mainly be used at altitudes where the flora is already tolerant to frost and therefore the trait would not confer any selective advantage via introgression to wild relatives. On the other hand, it was argued that if the frost-tolerant genes are established in weedy populations growing at lower altitudes these could spread to higher altitudes possibly eroding wild *Solanum* diversity. Participants noted that conventionally bred varieties resistant to frost are being used in the region without any noticeable effects on the environment. However, further studies on improved fitness following transfer of frost tolerance gene(s) was recommended. The experimental work should include the transfer of genes coding for frost tolerance to frost resistant as well as frost sensitive varieties.

Quality modifications

Traits which modify starch quality, protein content and dry matter of potatoes were considered to have negligible environmental impacts. In Latin America at the present time none of these traits were considered to be of major importance in the development of transgenic potatoes.

Concluding remarks

For some of the above mentioned traits there were environmental concerns that could not be addressed with present knowledge. No one at the meeting was aware of any environmental study on the release of a transgene (via gene flow to wild relatives) that demonstrated an increased fitness/weediness in plants. Therefore it was strongly recommended that studies with transgenic traits that confer increased fitness have to be performed in order to predict future situations and to better quantify possible risks. Participants argued that the whole world benefits from the utilization of germplasm from the centers of origin and that there is international concern for keeping this biodiversity. Therefore, studies on the impact of the use of transgenes in these environments should be an international responsibility. This is particularly important at a time when Latin American governments are less and less able to support these kinds of studies and the widespread use of transgenic plants will be difficult to control. However, the perceived risks should not be an impediment for transgenic potato research, or small restricted field trials, where effective risk management efforts can be implemented.

In summary, many countries will make decisions on whether they will or will not introduce transgenic plants based on their own needs. The view taken by many at the workshop was that it is a matter of balancing environmental concerns with potential benefits. The benefits will in many cases outweigh the perceived risks, but both need to be conscientiously and systematically evaluated. To do so, more information is needed on the environmental impacts of transgenic traits in specific regions of Latin America. Scientifically based estimates resulting from such impact studies could facilitate the safe deployment of transgenic potatoes in the region.

Discussion:

Q: Is the preservation of biodiversity being imposed on the governments of Latin American countries?

R: Being compelled to preserve local and regional biodiversity may, in effect, force Latin American governments to restrain from approving the commercial use of genetically engineered crops. This position may present a commercial advantage to the proprietors outside of Latin America of genetically engineered plants if alternatives developed in Latin America can not be approved.

Q: Who will fund scientific studies?

R: Already, there is less and less money in Latin America available for public research. Governments are cutting back on their portion of research funds and more money is derived from private companies. If this continues, nearly all research will be directed toward development of new products and little to environmental and social effects. Since centers of agricultural diversity are of global importance there should also be funding from outside Latin America for research on environmental impacts of transgenic crops, including potato, in centers of origin.

FOOTNOTES TO TABLE 1

1. Relative benefit is based on estimates of Low, Medium or High. Distinctions between the small farmer (SF) and large farmer (LF) users were made where possible: = indicates equal benefit; >> indicates greater benefit for the antecedent.
2. The nature of the concerns have been described in this chapter and earlier. The determinations listed here were based on considerations for the commercialization stage (large-scale) of transgenic crop use. For field trials, it was thought that environmental concerns could be sufficiently addressed by appropriate confinement measures.
3. These traits probably confer little additional fitness to the host; resistance genes are already present in wild *Solanum* populations; wild species are able to flower with bacterial infection.
4. This trait will probably confer little additional fitness to the host. Insect resistance development to pesticidal proteins may be controlled through agricultural management practices or, naturally, by the availability of alternative host plants.
5. Possible displacement of wild relatives due to increased fitness of specific *Solanum* species.
6. The use of male sterile, transgenic varieties was a general precautionary measure recommended for consideration in all commercial plant lines.
7. A study of possible disruptive ecological effects in different ecosystems (Andean, tropical) following a spray of *Bacillus thuringiensis* was suggested.

Table 1. Summary of group discussions on the environmental concerns and potential benefits of transgenic potatoes. The following assumptions were made prior to the deliberations: Transgenic potatoes will be commercialized in Latin America and there will be a gene flow to interfertile species.

Traits	**Country priority**	**Relative benefits/ to Whom**[1]	**Level of concerns**[2]	**Recommended control actions**[2]
Resistance to virus and bacteria	All countries Viruses (PVX, PVY, PLRV); Bacterial soft rot and bacterial wilt (*Erwinia*)	High to medium benefits. Lowering of seed production cost and better seed sanitary conditions Benefit: SF>>LF	Low to medium[3]	Male sterile lines should be used in centers of *Solanum* diversity to limit sexual outcrossing to wild relatives[6]
Resistance to insect pests	All countries Lepidoptera and Diptera (e.g. Potato Tuber Moth)	High benefits for environment and human health, less use of insecticides Benefit: SF = LF	Low to medium[4]	In order to better evaluate and, where possible, to quantify environmental risks, additional research needs to be done to determine the impacts of traits with a potential to increase fitness. Male sterile lines should be used in centers of *Solanum* diversity[7]
Resistance to fungi	All countries. Major problem is late blight (*Phytoptera infestans*)	High benefits (Less use of fungicides) Benefit: SF = LF	High in areas with wild *Solanum* populations[5]	See above
Tolerance to stress (frost and salt)	Frost: Argentina and Andean Countries Salt: Cuba and Peru	High benefit. Benefit: Especially farmers cultivating on marginal land	High in areas with wild *Solanum* populations[5]	See above
Altered starch/ protein quality	Argentina, Chile, Peru and Venezuela	Medium to low benefits Benefit: LF>>SF	Low	No specific action was recommended

Chapter 7
Field Testing of Transgenic Potatoes in the United States

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Introduction

The aboriginal home of cultivated potatoes is the South American Andes. Here we find a wide range of wild potatoes, cultivated potatoes and hybrids of various ploidy levels. The genus *Solanum* consists of about 2000 species. Within this genus, the section Petota, also known as section Tuberarium, includes the tuber-bearing members, of which the cultivated potato is best known. The wild species of the section Petota, numbering about 180, are prominent in the Peruvian and Bolivian Andes. They have been subject to repeated germplasm collection expeditions and still represent a rich source of diversity in breeding programs.

South America is not the world center of potato production. Most potatoes are grown as annual crops in temperate climates equivalent to the mountains of tropical areas. The major world producers, in order of production, are the former USSR, Poland, United States, Germany, and France. In the United States, potato production is concentrated in the northern tier of the country. In certain agricultural settings, mild winter conditions may permit potato seedlings to volunteer from tubers left unharvested the previous growing season. It is possible, then, for potatoes to grow as a perennial. This is the general case in South America.

Potato genetics make it difficult to breed and develop a menu of parental lines. The potato has a series of ploidy levels, based on a haploid number of 12, ranging from diploid (2N=24) to hexaploid (2N=72), and including triploids, tetraploids, and pentaploids. The cultivated potatoes (*Solanum tuberosum*) in the United States are tetraploids (4N=48). The fact that potatoes are vegetatively propagated, in combination with their relative low market price, removes the incentive to produce new lines. However, the need for new traits in potatoes remains valid. These traits include resistance to herbivorous insect pests, storage insects, or pathogens, and improved quality and nutrition. Plant breeders are frustrated because, for some necessities, new sources of resistance can not be found. This is the case for the Colorado Potato Beetle (CPB), *Leptinotarsa decemlineata*.

In the United States, potatoes are seasonally sprayed repeatedly with numerous pesticides and, after cotton, potato is the second most sprayed crop. CPB is the most damaging pest of the North American potato crop. In some parts of the US, infestations of the CPB result in complete production losses. Currently, growers depend primarily upon chemical pesticides to control the insect. The net result is that some growers must chose an alternative crop that obviously is not a host of the CPB. Biotechnology, the magic bullet to some, offers other alternatives to these necessities. The techniques of biotechnology have allowed the incorporation of new sources of resistance that would otherwise be unattainable and transgenic potato plants have been created.

Field testing of transgenic potatoes

In the United States, certain genetically engineered plants are regulated by the Animal & Plant Health Inspection Service (APHIS).

APHIS has had regulatory oversight of transgenic plants since 1988. It has issued over 1200 permits for field tests of transgenic plants. A permit may allow several field tests in several regions and in more than one season. The largest number of field trials for transgenic crops has occurred in the United States.

The USDA's view on transgenic plants approximates those of crops obtained through traditional methods. The framework to evaluate transgenic plants has therefore been developed using criteria for breeder or registered seed.

Initially, APHIS held a conservative view on the conduct of these tests. APHIS used data, methodologies, and other field parameters for producing hybrid and certified seed as the framework to evaluate field tests of transgenic plants. In retrospect, this was the correct approach. It supported the *a priori* view that the products of biotechnology were not sufficiently different than those produced by traditional methods to warrant special considerations.

Most potatoes are not sown from true seed (botanical seed). This fact permits a greater degree of discretion in managing field trials of transgenic potatoes. Presumably, a field trial of transgenic potatoes will be allowed to flower openly. Potato pollen arriving from within or outside the field test that is able to fertilize receptive flowers of the transgenic plants will have no consequence because the ensuing seed will not be used in future plantings. In the United States, potato is sown using pieces of the tuber containing at least one eye (lateral buds). The potatoes of commerce are all reproduced vegetatively, as clones. The source of these potato pieces are plants grown under strict agronomic conditions because they are susceptible to pathogens transmitted via tubers. Potato plants are notorious for sterility, both male and female, which causes difficulties in breeding. Most commercial cultivars in North America are male-sterile. Exercising good agronomic practices can ensure 100% pure growing material. In the United States, potatoes are grown as monoculture row crops of a single clone. The supply of seed is strictly monitored for purity and quarantine reasons.

Discussion:

Q: How do national governments with little expertise in regulating genetically engineered plants, especially in the areas of risk assessment and risk management, gain from the experience of others?

R: The USDA has provided material and indirect financial support to various countries (including many in Latin America) on this issue, but, in this period of mandatory budget reductions, this assistance will diminish. However, it's not necessary to establish *de novo* regulatory mechanisms for the products of biotechnology. Products should be considered to be similar to those developed by traditional breeding methods. Acceptance of this premise facilitates the oversight within existing national plant health regulations. Regional cooperation, such as is being done by Canada, the United States and Mexico within the North American Plant Protection Organisation should help alleviate scarcity in human, financial, and material resources. Assistance with expertise from independent organizations, such as the Biotechnology Advisory Commission (Sweden) is also a possibility.

In general, the accepted agronomic practices of the crop are used to conduct field tests of transgenic plants in the United States. A sufficient buffer zone is built into the field test to minimize cross-pollination with possible compatible plants beyond the test area. The best measure of effective separation distances are those recommended by plant breeders to obtain nearly-pure seed. An acceptable alternative may be beginning a field test earlier or later than natural conditions dictate to avoid matching the flowering periods of the experimental plants with those of indigenous compatible plants. For obvious reasons, cross-pollinating plants require greater separation distances than self-pollinating plants or vegetatively propagated plants.

Determination for non-regulated status

The USDA's regulatory oversight of transgenic plants allows "deregulation." That is not to say that the process allows immediate commercial sale of newly developed varieties. Approvals from the EPA and FDA may still be required. The principal criteria for exemption from regulation are that the plants:

- must exhibit no plant pathogenic properties;
- are no more likely to become a weed than nontransgenic cultivars which could potentially be developed by traditional breeding techniques;
- are unlikely to increase the weediness potential of any other cultivated plant or native wild species with which they can interbreed;
- should not cause damage to raw or processed agricultural commodities;
- are unlikely to harm other organisms beneficial to agriculture or adversely impact the ability to control non-target insect pests; and
- should pose no greater threat than the widely practiced method of applying insecticides to control the target pest (i.e. CPB) on potatoes.

It seems unlikely that a hybrid will become a weed. Currently, single transgenes are being evaluated and a single gene would not make a plant that has no weedy characteristics into a weed. Neither would a weedy characteristic be exacerbated.

Discussion:

Q: How elaborate must an experimental design be in order to adequately study the environmental effects following commercialization of a transgenic plant?

R: Besides the difficulty in addressing this question, there is an overriding difficulty of raising funds for research to create the best monitoring strategies. Therefore it may be more cost effective to monitor for environmental effects from already commercialized crops or where large field trials have been performed. A discovery of an unfavorable environmental effect will provide focus for monitoring of other releases which, in its turn, may facilitate action at an early stage. Sharing experience from field tests in Latin America and in other countries is important. It also applies to biosafety regulations. Biosafety committees from different countries should coordinate resources and establish cooperative programs to monitor transgenic material released on a large-scale*.

* Large-scale trials have been conducted in China. European scientists are presently collaborating with Chinese authorities to perform post planting surveys.

Introduction of insect resistant potatoes in the United States

In 1995 APHIS determined that transgenic potatoes resistant to CPB do not represent a plant pest risk. Therefore these transgenic potato lines (produced by Monsanto) will no longer be regulated under the Federal Plant Pest Act. Below are some general remarks on potential benefits and some environmental concerns.

Potential benefits

The Environmental Protection Agency (EPA) determined that transgenic plants producing the delta endotoxin of *Bacillus thuringiensis* ssp. *tenebrionis* to control the CPB provides a number of benefits.

Firstly, *B. thuringiensis* plant pesticides reduce the use of conventional chemical pesticides in potato production. No foliar chemical insecticides are needed for CPB control because

B. thuringiensis plant pesticides provide season-long control. This leads to the avoidance of hazards associated with conventional chemical pesticides including accidental release or exposure during shipping, storage, mixing and loading, application, and container disposal. This would also lead to the reduction of chemical pesticide concentrations in ground water in potato growing regions which have soil types predisposed to leaching. Secondly, *B. thuringiensis* plant pesticides produced by potatoes are more efficacious for CPB control than currently registered pesticides and non-chemical alternatives. This may induce an enhancement of IPM programs through increased biological control. Thirdly, the endotoxins are non-toxic to non-target organisms. Finally, there is a potential reduction of input costs for large and small farms alike.

In summary, the EPA found that production of potatoes containing *Bt*-endotoxin has the potential to provide a commercial crop of potatoes requiring fewer pounds and fewer applications of chemical pesticides, thereby saving growers' costs and reducing risks to humans and the environment.

The Food and Drug Administration (FDA) will be responsible for determining food allergenicity and toxicity. Although not the responsibility of the USDA, a company may utilize information gathered during the field trial phase to address biosafety issues raised by the EPA and FDA.

Development of resistance and resistance management

One of the most commonly expressed concerns relating to the large-scale application of CPB resistant potatoes is the development of *Bt* based insect resistance. Resistant herbivorous insect populations may arise from the pressure exerted by these transgenic plants. This fact is not disputed. Under debate, however, is how and when. Both of these questions may be made irrelevant if government, industry, and growers take certain precautions: judicious use of insecticides; Integrated Pest Management (IPM); refugia; crop rotation; flaming, trap plots; and temporal, tissue specific, or inducible expression of transgenes. To date, these practices are not used consistently and it is unusual when they are sufficient to replace all insecticide use against the CPB. The USDA acknowledges the potential for resistance to develop in insect pests. In many areas of the United States there are no currently registered insecticides that will control the adult CPB effectively. The use of pesticides to control the CPB have led to development of resistance in other pests such as aphids.

Companies such as Monsanto have developed resistance management plans for the CPB based on current information. However, there must be a commitment to refine these plans as new information is gathered. Specifically, there should be:

- continued refinement of information on the reproductive strategies of CPB with respect to gene flow, particularly regarding adult movement, larval movement, and behavioral responses including mating studies;
- continued refinement of optimal refugia strategies;
- continued development of specific monitoring plans including sites to be sampled, timetables for development, education of growers on sampling for resistance, collecting specimens to evaluate for resistance, and providing specific recommendations on how to eradicate resistant individuals to prevent survival of a resistant population;

- continued development of a data base to store monitoring information on the use of the genetically modified potatoes and facilitate correlation of resistance reports with the use sites;
- development of a discriminating dose assay;
- continued development of educational materials, in addition to technical bulletins, for growers describing how to cultivate genetically modified potatoes, monitor for resistance, and, if necessary use other insecticides;
- continued refinement of IPM recommendations such as crop rotation at the local level; and
- continued development of novel CPB control mechanisms with different modes of action.

Chapter 8
Participants' Recommendations*

Today, most countries in Latin America have research programs to construct genetically modified potatoes incorporating a variety of traits (e.g. pest resistance and frost tolerance). The countries are at various stages in the establishment of regulatory guideline mechanisms. Whether or not transgenic potatoes will be introduced as a local variety is in many countries a question of need. The participants felt that environmental concerns with a particular transgenic trait have to be balanced with potential benefits of improved varieties. In many cases the benefits will outweigh the perceived risks. The group recognized that gene flow from transgenic potatoes to their wild relatives is a much more relevant issue in Latin America than elsewhere. However, there have been too few studies on what environmental impact transgenic traits have when they are outcrossed into wild populations. Concerns were raised that traits conferring increased fitness to their hosts would outcross to the wild *Solanum* populations, thus changing growing patterns. Therefore, research on the environmental effects of gene flow specific to centers of diversity to better quantify environmental impact was recommended. The results from such experimentation could facilitate the safe development of transgenic plants in regional and national breeding programs. In Latin America however, there has been a limited amount of studies, partly due to lack of public funding. Many felt that the international community should help fund monitoring and research since centers of diversity are of global importance. To this end, the establishment of collaborative research and monitoring programs as well as information sharing mechanisms were called for. In order to provide input to future discussions and regional activities, the participants agreed on a number of recommendations, as follows.

1. The international community is urged to fund research activities that will result in scientifically substantiated estimates of the environmental impact of transgenic crops in centers of origin.

2. To quantify environmental impacts, additional research should be done in Latin America on traits considered to have a neutral, low or high fitness value and performed in a variety of environments.

3. All researchers and developers are strongly urged to consider the issues of biodiversity and the potential impacts of undesirable traits from gene flow.

4. Researchers and developers are urged to consider the use of male-sterile varieties of potatoes at the commercial stage.

5. Efforts should be made to identify production regions where related wild *Solanum* species are located. Data already

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* The recommendations are the result of workshop discussions combined with comments from all participants and do not necessarily reflect the view of the Biotechnology Advisory Commission or the Inter-American Institute for Cooperation on Agriculture.

available on existing species should be compiled and made easily accessible.

6. To assess environmental impacts, more effort needs to be directed towards establishing follow-up surveys after releases of genetically modified organisms.

7. Networking opportunities need to be established that will facilitate information exchange and encourage joint, collaborative research programs.

8. The need for uniformity of regulations across countries is recognized and recommendations from previous meetings articulating this view are supported.

9. Training should be made available to and assessed by all countries anticipating evaluation of release of genetically modified crops, especially where existing technical capacity is low.

10. In evaluation processes, there is a need for multidisciplinary cooperation involving scientists such as ecologists, population geneticists and molecular biologists.

11. Scientists involved in transgenic crop research should be cognisant of biosafety issues and adhere to good developmental practices.

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The Workshop

International and national research laboratories in Latin America have now begun testing transgenic plants developed by their own scientists. More than ten local research groups are developing transgenic potatoes, making it the most popular transgenic crop in the region. The biosafety assessment of transgenic potatoes represents a special challenge to Latin American regulatory officers since very few environmental impact assessments have been done in the region. Because Latin America and in particular the Andean region is the center of potato diversity, the situation is different from that in Europe and the United States. The countries in the region must respond to questions about the likelihood of gene spread from transgenic potatoes to their wild relatives and an uncertainty about possible impacts on genetic crop diversity. The BAC/IICA workshop was designed to take advantage of the latest information and consider both small and large-scale stages of crop development. Scientists and regulatory officers from all over Latin America gathered to discuss the issues with invited resource persons. These workshop proceedings reflect the current state of thinking about the environmental impact of transgenic plants in centers of origin from the Latin American perspective.

Biotechnology Advisory Commission (BAC)

is an independent advisory body with headquarters at Stockholm Environment Institute, Sweden. Upon request from government authorities the international experts of the Commission review biotechnology proposals and provide independent, impartial advice on issues of biosafety. Since its establishment in 1993 the BAC has been involved in a number of international activities aimed at capacity building for biosafety implementation in developing countries.

Inter-American Institute for Cooperation on Agriculture (IICA)

is an intergovernmental technical cooperation agency composed of 33 member states in the Americas. IICA has its headquarters in San José, Costa Rica and has a total staff of almost 500 international and national specialist, and offices throughout the region. It was originally established in 1942 as a tropical agriculture studies and research center. Since then it has facilitated and supported cooperation between its member states on agricultural development and rural well being.

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