



ISB NEWS REPORT

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In This Issue:

Colombia Biosafety Council Takes on Training	1
Noble Foundation, NCGR Release First Round of Medicago Truncatula ESTs	3
Metabolically Modified Rice Exhibits Superior Photosynthesis and Yield	4
Chloroplast Expression of Therapeutic Proteins Proves Highly Efficient	5
Insect Resistance and the Future of Bt Transgenic Plants ..	6
Is RIDL the Answer?	8
The Monarch Report from USDA	10
Congressional Report on Biotechnology	10
Academia Provides Answers to Biotechnology Questions	10
Scientists Unite for Biotechnology	10
Upcoming Meetings	11



NEWS AND NOTES

COLOMBIA BIOSAFETY COUNCIL TAKES ON TRAINING

Researchers working on the application of biotechnology to agriculture in the developing world often mention a vegetable you'll probably never see in your average supermarket, say, in Ames, Iowa: cassava. "You don't see Monsanto or other multinationals researching cassava," say the scientists. "This is so despite the key role this crop plays in the diets and livelihood of some 500 million people in Asia, Africa, and Latin America," they explain.

This comment—nearly transformed into aphorism through repetition—was heard again at a first-ever workshop on biosafety for members of Colombia's Biosafety Council, held from April 12-15 at the International Center for Tropical Agriculture (CIAT), a CGIAR center near Cali. And the comment is mentioned here because it serves to underscore a basic fact: biotechnology in the developing world is slowly and surely acquiring its own identity. This is evidenced by the laws, decrees, or resolutions that are creating councils and commissions charged with regulating biotechnology in developing nations, the training these councils and commissions are seeking, and the research they sanction or prohibit. The workshop held at CIAT brought this point home.

Rodrigo Artunduaga, coordinator for the Genetic Resources and Biosafety Unit at Colombia's national agricultural research institute—and head of the eleven-member Biosafety Council—explained why a workshop was needed in the first place. "Since we were created by a government resolution 16 months and nine meetings ago, we've had nine applications for greenhouse or field trials of transgenic crops, and reaching consensus has been difficult," said Artunduaga. "What we've found is that several members of the council are simply lacking in the background necessary for evaluating these applications, and we want to make sure there are no problems with authorizations."

Colombia's council was not the first body created to regulate biotechnology in the Latin American and Caribbean region (LAC)—Argentina holds that honor, having set up their commission in 1991. Nor will it be the last. Its eleven members include representatives from the Ministries of Health, the Environment, and Agriculture, as well as a farming organization, an agricultural NGO, a seed company, a multinational (DuPont—required to withdraw if the company submits an application), a microbiology professor, and three members of the national agricultural research institute. In this panorama, it should be no surprise that certain members "have some level of ignorance about transgenics," as the council head put it.

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And so the workshop spanned from "Genes: What are They?" to a talk on "What are the challenges for biotechnology in the tropics and what role should biotechnology play?" Zaida Lentini, plant geneticist and workshop organizer, called the event "overdue, but welcome." She noted that the work of Colombia's council, and that of other similar bodies in the region, is somewhat complicated by "a tendency to import health and environment arguments and debates from Europe, and agricultural research objectives from the United States."

In LAC, for example, a key environmental issue in years to come will be gene flow, since the region includes five of the world's twelve centers of origin and domestication of species. Examples include corn, with ancestors in Mexico, and cassava, whose center of origin is thought to be Brazil. Agricultural research objectives unique to the region include the work of Dr. Lentini herself, whose research includes a transgenic variety of rice resistant to the hoja blanca virus—an endemic pest that periodically wreaks havoc on this staple crop.

In fact, Dr. Lentini's research program is one of three at CIAT that have submitted applications to the Biosafety Council for greenhouse and field trials. When asked about the possible conflict of interest involved in having sought out CIAT to offer the workshop, Dr. Artunduaga said that consensus had been reached on the applications in the council's most recent meeting, even though they hadn't been formally approved. Dr. Lentini underscored that the workshop's content in no way referred to CIAT's pending applications.

The other two applications CIAT has before the council are for greenhouse studies of transgenic plants carrying marker genes, with tropical pastures and—surprise—cassava. The latter is being done with an eye towards insect resistance. And while all three—as well as the other six applications—are oriented towards agronomy performance, Artunduaga and Lentini both see gene flow experiments as a next frontier of sorts, both in Colombia and in the tropics in general. "I'd like to be studying this right now," said Dr. Lentini, "but first things first." Dr. Artunduaga said that "in the tropics, we must further our knowledge of ecosystems, expression and stability of incorporated genes, the botany and geographical distribution of those species of which the region is a center of origin, and the technical basis for risk assessment and risk management."

Of course, the above will require training, and lots of it. This is why Dr. Artunduaga also called legislation "only the first step" towards lifting biotechnology off the ground in Latin America and the developing world in general. "After that," said the head of Colombia's Biosafety Council, "you have to develop a critical mass of experts within each country to study and monitor the benefits and effects of transgenic crops. It makes no sense to have laws,



decrees, or whatever, which require a pile of data, if no one is capable of interpreting the data.”

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NOBLE FOUNDATION, NCGR RELEASE FIRST ROUND OF *MEDICAGO TRUNCATULA* ESTs

The National Center for Genome Resources and the Samuel Roberts Noble Foundation have released new EST (expressed sequence tag) data from a collaboration studying *Medicago truncatula*, a “model” legume used in genetic studies.

The organizations have released 14,634 expressed gene sequences. The new sequences, which nearly double the amount of publicly available *M. truncatula* EST data, are accessible through the Noble Foundation and NCGR Web sites and were released by GenBank today.

Scientists from NCGR and Noble are working together to identify novel expressed gene sequences; such EST data will be useful to researchers around the world. The data release is the first in a series expected over the next 18 months. Analysis of these EST data ultimately may lead to improvements in legume crops.

“We’re excited about our results and our opportunity to contribute to the model legume scientific community,” said Gregory May, Ph.D., project leader for Noble. “Genomics programs such as ours can generate information that *Arabidopsis* simply can’t provide because *M. truncatula*, and legumes in general, have their own unique metabolic pathways and plant-microbe interactions that warrant further study.”

The *M. truncatula* research community, including National Science Foundation projects and international groups such as those in France, will continue to contribute to the overall genomic research of the plant, making it a stronger model system. The Noble and NCGR scientists credited this community and teamwork within their organizations for the progress to date.

M. truncatula and its better-known relatives, alfalfa and soybean, are members of the legume family, one of the

most important groups of plants. Legumes are a crucial source of protein for humans and forage food for animals. *M. truncatula* is closely related to alfalfa (*M. sativa*), and the results of the collaborative project will therefore be directly applicable to the improvement of one of the world’s major forage crops. *M. truncatula* is a particularly good plant for study because, unlike alfalfa, it has a relatively small genome and is amenable to efficient genetic and molecular analysis.

The *M. truncatula* collaboration allows Noble to focus on data generation and NCGR to focus on structuring data analysis. Noble conducts extensive research in agricultural sciences, and NCGR’s specialty is bioinformatics, which employs mathematics and computer science to analyze biological data.

Data and detail about the *M. truncatula* project are available at <http://www.ncgr.org/research/mgi/> and <http://www.noble.org/medicago/>. Medicago sequences also are accessible through NCGR’s Genome Sequence DataBase.

About NCGR

The National Center for Genome Resources (<http://www.ncgr.org>) is an independent, nonprofit life sciences research institute working at the interface of biology, computer science and mathematics (bioinformatics). By conducting and facilitating research the Center contributes to the improvement of global nutrition, health and environmental well-being.

About Noble Foundation

The Noble Foundation (<http://www.noble.org>) is a privately funded, nonprofit organization headquartered in Ardmore, Okla. The Foundation conducts agricultural and plant biology research; provides grants to numerous other charitable and educational organizations; and assists farmers and ranchers through educational and consultative programs.

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PLANT RESEARCH

METABOLICALLY MODIFIED RICE EXHIBITS SUPERIOR PHOTOSYNTHESIS AND YIELD

Rice, a staple food crop for nearly half of the world's six billion population, is a key target for genetic engineering aiming to develop improved varieties to feed the world's burgeoning population. International Rice Research Institute experts say rapid population growth, especially in developing countries, has caught up with advances in cereal yields in the past three and a half decades. To meet the demand, a 40% increase in rice yield is needed by 2020. Farmers must consistently produce an extra 6.7 million tons of unmilled rice every year using less land and less water just to maintain current nutrition levels. Considering the fact that the current world rice production has leveled off at 590 million tons per year, this represents a more than 10% increase per year. Conventional breeding programs can deliver only small increments in yield, mostly in the range of a few percent. By introducing the photosynthesis genes of maize into rice, our research team including Professor Makoto Matsuoka, Nagoya University and Dr. Mitsue Miyao, National Institute of Agrobiological Resources, Japan has recently demonstrated that the new rice strains we have produced could boost photosynthesis and grain yield by up to 35%. This technology may help in filling the bowl, thus combating hunger.

Most of our conventional crops, including rice and wheat, assimilate atmospheric CO₂ by the C₃ pathway of photosynthesis, which takes place in the mesophyll cells of leaves. Photosynthetically, these plants are underachievers because, on the one hand, they assimilate atmospheric CO₂ into sugars but, on the other hand, part of the potential for sugar production is lost by respiration in daylight, releasing CO₂ into the atmosphere, a wasteful process termed photorespiration. This is due to the dual function of the key photosynthetic enzyme, ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco). High CO₂ favors the carboxylase reaction and thus net photosynthesis; whereas high O₂ promotes the oxygenase reaction leading to photorespiration. When plants first evolved, photorespiration was not a problem because the atmosphere then was high in CO₂ and low in O₂. As a byproduct of photosynthesis, O₂ accumulated in the atmosphere and reached the present level a million years ago. Current atmospheric CO₂ levels limit photosynthesis in C₃ plants. Furthermore, photorespiration reduces net carbon gain and productivity of C₃ plants by as much as 40%. This renders C₃ plants less competitive in

certain environments. In contrast, with some modifications in leaf anatomy, some tropical species (e.g., maize and sugarcane) have evolved a biochemical "CO₂ pump," the C₄ pathway of photosynthesis, to concentrate atmospheric CO₂ in the leaf and thus overcome photorespiration. Therefore, C₄ plants exhibit many desirable agronomic traits: high rate of photosynthesis, fast growth, and high efficiency in water and mineral use.

Unfortunately, there are no closely related C₃ and C₄ crops that we can use to transfer the C₄ traits to C₃ crops by a traditional breeding approach. Thus, our research team has been interested in engineering the C₄ traits in rice to enhance its productivity. In engineering C₄ photosynthesis, there are two important components to be considered: the biochemical pathway (enzymes) and the specialized leaf structure. The coordination of two specialized leaf cells in C₄ leaves, namely mesophyll and bundle sheath cells (together termed Kranz leaf anatomy), is important for pathway function. The enzymes and their corresponding genes involved in the C₄ pathway of photosynthesis have been characterized. However, very little is known about the molecular mechanisms controlling the differentiation of Kranz leaf anatomy in C₄ plants. Therefore, our first goal was to engineer the key enzymes involved in C₄ photosynthesis in rice without Kranz leaf anatomy. At first thought, one may argue that rice plants thus engineered may not be very efficient in concentrating CO₂ in the leaf, as Rubisco is located in the chloroplasts of the inner bundle sheath cells in C₄ leaves. The cell wall of these well-differentiated inner cells has special constituents that prevent CO₂ from leaking out of the leaf. However, in nature, a primitive aquatic plant, *Hydrilla verticillata*, is known to be able to use a simplified version of the C₄ pathway (without Kranz leaf anatomy) to concentrate CO₂ and eliminate the wasteful photorespiration process¹.

Using an *Agrobacterium*-mediated transformation system, we have independently introduced into rice three maize genes encoding the C₄ photosynthetic pathway enzymes: phosphoenolpyruvate carboxylase (PEPC); pyruvate, orthophosphate dikinase (PPDK); and NADP-malic enzyme (ME)². The transgenic rice plants express high levels of these genes and the maize enzymes remain active. Most importantly, PEPC and PPDK transgenic rice plants exhibit higher photosynthetic capacity than untransformed plants, mainly due to an increased stomatal conductance (i.e., more atmospheric CO₂ becomes available for fixation)³. Preliminary field trials conducted in China and Korea also show 10-30% and 30-35% increases in grain yield for PEPC and PPDK transgenic rice plants, respectively. These results were totally unexpected since only one



of the maize C_4 pathway enzymes is being elevated in the transgenic rice plants and one would not expect this would be sufficient to concentrate CO_2 as in a typical C_4 plant. Indeed, direct fixation of atmospheric CO_2 via these individual enzymes remains low in the transgenic rice plants. However, we believe increased synthesis of organic solutes (e.g., malate) by the enzymes in the guard cells may be responsible for the enhanced conductance of CO_2 by the stomates since stomates open by pumping up their levels of solutes. In this regard, it is interesting to note that increased yields in new wheat cultivars, developed by CIMMYT in the past 30 years, are attributed to increased photosynthetic capacity, which is associated with an elevated stomatal conductance to CO_2 diffusion.

A further enhancement of the photosynthetic capacity of rice will require engineering a limited C_4 pathway of photosynthesis by simultaneously expressing the three previously mentioned key enzymes in proper cellular compartments. Using a conventional breeding approach, we have produced hybrids among the three transgenic lines. Tests for their photosynthetic and growth performance are underway. Ultimately, for most efficient operation of the pathway to concentrate CO_2 around Rubisco in the leaf, the concomitant installation of Kranz leaf anatomy will be essential. More work is needed in order to convert the less efficient C_3 rice to a more efficient " C_4 rice."

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CHLOROPLAST EXPRESSION OF THERAPEUTIC PROTEINS PROVES HIGHLY EFFICIENT

The production of industrial and pharmaceutical proteins is traditionally carried out in yeast and animal cell systems. However, the use of transgenic plants for production of human therapeutic proteins may offer distinct advantages—high output at relatively low cost and maintenance requirements, and a reduced risk of mammalian viral contamination. Recombinant antibodies and hemoglobin are already being faithfully produced in plant cell cultures. Animal proteins for use as vaccines and nutritional supplements, such as alpha-lactalbumin, are proven to work well when expressed in field plants.

The feasibility of using plastids for production of human proteins has been demonstrated by a research team at Monsanto led by Jeffrey Staub. In a recent article in *Nature Biotechnology*, Staub et al. report the expression of biologically active human somatotropin (hST) in high concentrations in tobacco chloroplasts¹. Human somatotropin is used therapeutically for treatment of hypopituitary dwarfism in children, Turner syndrome, chronic renal failure, and HIV wasting syndrome. Chloroplasts have been successfully used for production of recombinant bacterial proteins, however the production of high concentrations of mammalian proteins has not been previously reported. Chloroplast expression systems may confer advantages over nuclear expression—very high expression levels of protein, reduced risk of transgene escape (as chloroplasts are rarely transmitted in pollen), and predictable, uniform, and stable gene expression not subject to gene silencing.

Staub et al. incorporated the hST gene into three plasmids: wrg4838, pMON38755, and pMON38794. Plasmids wrg4838 and pMON38755 utilized an upregulated plastid *psbA* promoter, and pMON38794 a constitutive chloroplast rRNA promoter (*Prm*), in order to maximize accumulation of hST in the chloroplasts. In the pituitary gland of humans, a signal peptide is cleaved from hST leaving phenylalanine as the N-terminal amino acid of the biologically active form. In order to duplicate this modification in the chloroplast, a yeast ubiquitin gene was fused to the hST gene in the pMON38755 and pMON38794 plasmids such that post-translational cleavage would yield an N-terminal phenylalanine on hST.

Their results showed the transplastomic Nt-4838, -38755, and -38794 lines accumulated 0.2%, >1% and ~7% total soluble protein, respectively, in the mature leaves of tissue culture derived plants. The 7% total soluble protein level

represents > 300-fold higher accumulation than that found using the nuclear transgenic approach, which indicates a substantial advantage of the chloroplast expression system. The ubiquitin-hST processing efficiency was confirmed by western blot tests, proving the utility of fusion protein method for facilitating protein processing in chloroplasts. Further testing on a rat lymphoma cell line that proliferates in the presence of hST indicated that the chloroplast-derived protein was as biologically active as naturally produced hST.

The bioengineering of genes into chloroplast rather than nuclear DNA may reduce the risk of horizontal gene transfer from crops to wild plants since plastids are not transmitted through pollen in most species. Staub found no hST accumulation when transplastomic lines were used as pollen donors in crosses with wild-type tobacco, which indicates strict maternal inheritance of plastid transgenes. Testing also showed that the transplastomic lines displayed normal growth and were fertile.

The chloroplast expression system as developed by Staub's group is highly efficient and demonstrates great potential for the large-scale production of transgenic cultures and field crops capable of producing large quantities of pharmaceutical proteins. In addition to the intended applications proposed by Staub et al., this system offers potential advantages to researchers wishing to produce crops with higher yields of other beneficial proteins as well.

Source

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INSECT RESISTANCE AND THE FUTURE OF BT TRANSGENIC PLANTS

Pest resistance to insecticides is a worldwide phenomenon and current estimates indicate that over 500 arthropod species have developed strains resistant to one or more pesticides. These cases of resistance are not limited to synthetic insecticides, but also include a wide range of 'natural products' including pathogens and insect growth regulators. Unfortunately, this also includes resistance to toxins from strains of the common bacterium, *Bacillus thuringiensis* (Bt). Bt products had been used for more than 40 years as insecticidal sprays without any evidence of resistance in field situations until a report from the Philippines indicated control failures of the diamondback moth (DBM), *Plutella xylostella*. Subsequent studies in Hawaii and Florida documented the genetic basis of resistance of Bt and further reports have documented control failures of Bt against DBM in other parts of the US, Japan, Central America and China. Laboratory populations of at least 10 species of moths, two species of beetles and four species of flies have been exposed to selection against Bt toxins and ten-fold increases in tolerance have occurred in nine of the 16 species¹. These findings warn of the possibility of insects developing resistance to transgenic insecticidal plants containing Bt toxins. Although there are no cases of insects developing resistance to Bt transgenic plants in the field, laboratory populations of Cry1A-resistant DBM have been shown to be able to survive on transgenic crucifers expressing high levels of Cry1Ac².

The development of resistance to Bt transgenic plants would negate the benefits of this new technology, now grown on ca. 11.8 million hectares worldwide. Some of the benefits of Bt plants can be illustrated with Bt cotton in the US. Since the commercialization of Bt cotton in 1996, insecticide sprays on cotton have been reduced by approximately 3.8 million liters of formulated product per year in the US and this has led to a significant reduction in the use of more hazardous organophosphate and pyrethroid insecticides³.

Can Bt plants be deployed so that resistance will be delayed or avoided? It has been suggested that Bt plants may, in fact, be more effective at managing resistance than Bt foliar sprays because one is able to regulate the dose more effectively in the plant than with a spray. While this may bode well for Bt plants, the question still remains about how to deploy them to reduce the likelihood of resistance developing. Various strategies have been proposed but the



only commercially available approach is the use of a high dose of a single gene, producing 25 times the toxin concentration needed to kill susceptible insects, in combination with a refuge. The refuge is composed of non-transgenic plants and is intended to generate sufficient numbers of susceptible insects to dilute resistant alleles, while at the same time allowing the non-transgenic plants to generate high yields. While this sounds like a good idea, there is considerable debate on the required size of a refuge. Presently, a 20% refuge is recommended for cotton and corn but some workers have called for refuges as large as 50%, if farmers are allowed to spray them. This allotment size presents a dilemma since farm profitability and reduction of pesticide use may come from larger proportions of transgenic crops, but long-term enjoyment of these benefits may be feasible only by limiting the percentage of the crops that are transgenic.

We have used DBM in combination with crucifers engineered to express a Cry1Ac toxin to study factors that influence the development of resistance⁴. In greenhouse trials we introduced DBM that had an initially low Cry1Ac resistance gene (R) frequency into cages with various ratios of Bt broccoli and non-Bt broccoli plants. The insect populations were allowed to cycle for several generations and then the larvae were tested for resistance. Pure stands of Bt-expressing plants (0% refuge) resulted in rapid development of highly resistant DBM populations, and increasing the size of the refuge delayed the development of resistance. Furthermore, the placement of the refuge plants significantly affected the development of resistance. When both plant types were mixed in a random spatial arrangement ('mixed seedling model') larvae were able to move between plant types. As they moved from refuge plants to Bt-expressing plants, they died and caused a decline in the number of susceptible alleles (S) in the overall population. This resulted in a more rapid development of resistance than when plants were separated by a distance that limited the movement of larvae.

We then took our studies into the field. For the first year of tests, we examined the effect of refuge size and refuge placement (mixed vs. separate refuges) on the distribution of the larvae within the plots as well as the level of resistance in DBM at the end of the season. Our results demonstrated that the cumulative number of larvae per plant on refuge plants through the season in the 20% mixed refuge was significantly lower (6.4 vs. 14.6) than the 20% separate refuge. This finding indicates that, as in our previous greenhouse experiments, a separate refuge is more effective at conserving the number of susceptible alleles. This is because larvae on these refuge plants will be more likely to survive to

adults (either SS or RS) that can mate with RR individuals and thereby reduce the number of RR offspring. This evidence supports the use of a separate refuge for Bt-transgenic crops susceptible to insects that can move between plants as larvae.

We then examined the effects of spraying plants in the 20% separate refuge and, as expected, spraying reduced the capacity of the refuge to dilute resistance in the larger field. Our results indicated that if the refuge were left unsprayed, it would give a larger number of susceptible insects a chance to survive. The short-term sacrifices of having relatively more insects in the unsprayed refuge would translate to seasonal reductions in resistance and in the total number of larvae per plot. However, the critical question is whether such populations would result in unacceptable crop losses, and the answer to this will depend on the particular crop/insect system and the techniques used to manage the insects in the refuge.

Our studies provided the first empirical data demonstrating the usefulness of the refuge strategy for Bt plants. However, they also indicate the need to effectively monitor and manage susceptible alleles on an individual field or farm basis as well as on an area-wide basis. Within an individual field or farm, treating the refuge with a highly effective insecticide may dilute the abundance of susceptible alleles to such an extent that the refuge becomes less effective unless there is substantial immigration of susceptible alleles from wild hosts or from surrounding non-Bt crops. On the other hand, growers may be reluctant to sacrifice a large number of refuge plants to insects just to maintain susceptible alleles. Critical experiments need to be performed in the specific insect/Bt crop system to determine the correct balance between conserving susceptible alleles while providing acceptable crop yields.

The theory of resistance management has the potential to work in the field for the first generation of insecticidal plants. New technologies under development for the second generation of plants include Bt expression modes that subject insects to selection pressure for specified periods of time, and in particular, plant parts by using inducible and/or tissue specific promoters. These techniques may allow for larger refuges for susceptible alleles both within the field and within a region while at the same time minimizing crop loss. Other options are also possible. Theoretical models suggest that pyramiding two dissimilar toxin genes in the same plant has the potential to delay the onset of resistance much more effectively than single-toxin plants released spatially or temporally and may require smaller refuges⁵. Other non-Bt genes may also aid in managing resistance to Bt crops.

The development and implementation of engineered insecticidal plants is currently in its infancy but is providing substantial benefits for growers and the environment. It is important that industry, public-sector scientists, and farmers work together to develop a second generation of technology and implementation strategies to ensure that insects do not rapidly develop resistance to Bt crops.

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INSECT RESEARCH

Is RIDL THE ANSWER?

Transgenic crops expressing insecticides are in large-scale use, though not without considerable controversy. Lagging rather behind is the use of transgenic insects to control unwanted insects. Insects can themselves serve as pest control agents when employed as either predators or parasites of the target insect. Using the Sterile Insect Technique (SIT), the target insect is itself modified to become its own control agent. Large numbers of sterile males are released into the wild where they compete for mates with wild males, thereby reducing the pest population numbers in the next generation. If sufficient numbers

of the sterile males are released over adequate time, the total eradication of the target species can be expected. The most spectacular success of SIT was seen with the eradication of the screwworm fly (*Cochliomyia hominivorax*) from southern US and Mexico, but it has been used with varying success against a wide range of species, not all of them insects. We have recently proposed a major modification to SIT, using transgenic insects engineered to carry a dominant, repressible, female-specific lethal genetic system¹.

The key advantages to using SIT are its extreme specificity and ability to eradicate the target species over a wide area. The specificity is inherent in the mechanism—only the target females will mate with the released sterile males—so even closely related species are unaffected. The possibility of total eradication is a little more complicated. First, SIT becomes more effective as the control program progresses and the ratio of released sterile males to wild fertile males increases. Second, it is extremely difficult for the target species to develop resistance to SIT. The system is simply based on the ability of males and females to seek out each other and mate. The only general mechanism for “resistance” is assortative mating, which occurs if wild females can discriminate between released sterile males and wild fertile males and then exclusively mate with wild males. This was indeed a problem in the early days of SIT, but is now overcome by regularly outbreeding the factory stock with individuals caught in the wild and quality control procedures to ensure that the factory stock maintains genetic diversity and mating performance. The possibility of eradicating a pest species over a wide area, as was done with the screwworm fly, is extremely attractive and is essentially impossible by any pesticide program, or even by other biocontrol methods.

Two major drawbacks to current SIT methods result from the negative effects of sterilization by irradiation and difficulties in sex-separation procedures. A sterilizing dose of irradiation (or chemicals) unavoidably reduces the fitness and competitive mating ability of the irradiated males relative to wild males. Furthermore, the costs of constructing, operating, and decommissioning the radiation source add significantly to the cost of the control program.

Sex separation is important because released females may be detrimental to the control program, causing, in many cases, exactly the kind of damage that the control program is trying to prevent. Separation of the sexes prior to release is easy to do under a microscope for small numbers, but on a factory scale it is a major problem. For example, the Guatemalan medfly facility produces about 250,000,000



sterile male medfly per week, so a robust and automated sex-separation mechanism is clearly required. In the case of medfly, sex-separation is achieved genetically by using a recessive temperature-sensitive lethal mutation on an autosome with a covering translocation on the Y chromosome. This means that a precise shift in temperature at the right time will kill the females but not the males. Related systems have been developed for a few other species. Unfortunately, these translocation-based systems are intrinsically unstable and also require a certain amount of luck, as well as a great deal of hard work, to develop. Furthermore they are completely non-portable—a system developed in one species cannot be transferred even to closely related species without essentially starting from scratch. Finally, the mutations and chromosome aberrations involved themselves have adverse consequences on the fitness of the flies carrying them.

We propose that all these problems can be overcome by using recombinant DNA technology to construct a strain of the target insect that is homozygous for a dominant, repressible, female-specific lethal genetic system. The lethality has to be repressible in the factory, or all the females would die and one could not grow the strain, and it has to be reliable in the wild. While temperature and other factors could be used to control the lethality, use of a dietary additive that is unavailable in the wild diet could provide the best option. Withdrawal of the repressor chemical from the diet in the last factory generation would be lethal to all the females, resulting in a single-sex male population. If these males were then sterilized by irradiation and released, we would have combined conventional SIT with an efficient genetic sexing mechanism.

This mechanism alone would be a major advance but we further suggest that in fact this single-sex male population can be released as a control agent *without* sterilization. We call this system Release of Insects carrying a Dominant Lethal (RIDL) as the males are not, strictly speaking, sterile. In this version of RIDL, the released males will compete for mates with wild males, as normal. Any wild female mating with a RIDL male will produce offspring, all of which carry one copy of the RIDL system. All her daughters will therefore die from the female-specific lethality. Her sons will survive, but as they carry one copy of the RIDL system, half of their daughters will die, and so on. Since reducing the number of females will clearly lead to a reduction in the total population in the next generation, this strategy controls, and may ultimately eradicate, the target population in very much the same way as SIT. In our paper we suggest a number of ways in which this basic RIDL strategy might be improved, for example making the

factory stock homozygous for the RIDL construct at two loci rather than one, so that 75% rather than 50% of the released males' granddaughters die.

RIDL gives a much simpler production system than SIT—sex-separation is a simple consequence of withdrawing the food additive from the final factory generation and no sterilization step is required. Furthermore, the released males do not carry any mutations or chromosome aberrations other than a simple insertion of an engineered transposon, and have not been irradiated, so their fitness and hence effectiveness should be better than that of conventional SIT males. The RIDL strain should be much easier to construct than classical genetic sexing strains, allowing this technique to be used in a wider range of species.

RIDL has some obvious limitations. In common with SIT, it requires mass-rearing of the target insect, can generally only be used over a large area, and is more expensive than chemical methods. Relative to SIT, RIDL is limited to species for which genetic transformation is possible. Transgenic methods for non-*Drosophila* insects have only recently been developed, but appear to be quite general in application, so this is likely not a major restriction.

A very real disadvantage could be political resistance to releasing large numbers of transgenic (rather than mutated and irradiated) males into the environment. The concern here is gene flow—can the transgene “escape” into a wild population and, if so, what would be the consequences? Clearly the RIDL transgene will enter the target population at quite high frequency—that's the intention! But what about other populations and species? Animals have extremely strong behavioral and genetic mechanisms preventing inter-specific gene flow by mating, so the risk of transfer to other species is very low. The crucial point, though, is that neither the whole RIDL system, nor any part of it, should confer any selective advantage on the recipient; indeed we expect it to be highly deleterious. Consequently, it will only be maintained in the target population by continuous release of factory-reared RIDL males and any rare “escapes” to other populations or species will be rapidly eliminated.

As well as presenting the RIDL concept, we demonstrate that the required genetic characteristics can be achieved using the fruit fly *Drosophila melanogaster*. *Drosophila* is the best-known and best-characterized insect genetic model (completion of the *Drosophila* genome sequence was recently announced in *Science*) but is not itself an agricultural pest or disease vector. Our system contained a

promoter driving a tetracycline-repressible transcription factor (tTa) and a “killer gene” under control of the tetracycline responsive element (tRe). Female-specificity can come from either the promoter or the killer gene and we demonstrate both versions. We deliberately avoided using any *Drosophila*-specific genetic tricks, so we are confident that our system can be translated to insects of agricultural or medical significance. We have encouraging results with medfly and the yellow fever mosquito (*Aedes aegypti*) and are considering a number of other species.

Source

Thomas DT, Donnelly CA, Wood RJ, and Alphey LS. 2000. Insect population control using a dominant, repressible, lethal genetic system. *Science* 287: 2474-2476.

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INTERNET NEWS

THE MONARCH REPORT FROM USDA

A 10-page fully illustrated report titled “Monarch Butterflies and Bt Corn: Lab Research & Field Realities” now is available on the Web:

<http://www.fooddialogue.com/monarch/index.html>.

The report summarizes recent research from the public sector that establishes Bt corn pollen will have minimal if any impact on monarch butterflies in nature. The report featuring research and quotes by scientists from nine universities, USDA Extension Service, and the USDA-ARS is designed to educate the public and for use in classrooms from grade school to universities.

CONGRESSIONAL REPORT ON BIOTECHNOLOGY

A comprehensive report on Benefits and Risks of Plant Biotechnology entitled “Seeds of Opportunity” is available at <http://www.house.gov/science>. This report was issued by the US Congressional Committee on Biotechnology on April 13, 2000 and is the outcome of a series of three hearings held by the Congressional Biotechnology Committee in Washington DC.

ACADEMIA PROVIDES ANSWERS TO BIOTECHNOLOGY QUESTIONS

An Ohio State committee consisting of biologists, plant breeders, agronomists, agricultural economists, animal scientists and others are answering questions on genetically modified organisms. Visitors can ask questions at the group’s Web site: <http://ohioline.ag.ohio-state.edu/gmo>. It also includes articles, FAQ’s (Frequently Asked Questions), and links to other GMO Web sites and articles.

At Purdue, a similar committee has been working to answer questions about GMO’s and biotechnology in general. “Agricultural Biotechnology: What’s all the fuss about?” is in the March, 2000 issue of the Purdue Agricultural Economics Report, at <http://www.agecon.purdue.edu/extensio/paer.htm>. Background information on Purdue’s biotechnology research can be found at <http://www.agcom.purdue.edu/AgCom/news/backgrd/biotech.html>.

SCIENTISTS UNITE FOR BIOTECHNOLOGY

Disparaging misinformation suffusing the debate surrounding biotechnology and genetically modified (GM) foods has prompted one scientist to fight back. The formidable energy of C. S. Prakash, Director of the Center for Plant Biotechnology Research at Tuskegee University and frequent contributor to the *ISB News Report*, is focused on countering media-driven dispersions cast at the biotechnology industry. To that aim, Prakash has initiated a Web site, *AgBioWorld.org*, devoted to bringing information about agricultural bioengineering and GM food to the developing world. “We in the scientific community felt it necessary to counteract the baseless attacks so often being made on biotechnology and genetically modified foods,” opined Prakash at the BIO 2000 conference last March in Boston.

AgBioWorld.org is the home of the widely heralded Declaration of Scientists In Support Of Agricultural Biotechnology, a petition written by Prakash and a few colleagues that expresses strong “support for the use of recombinant DNA as a potent tool for the achievement of a productive and sustainable agricultural system.” The growing list of 2100+ scientists adding their names to the petition since its inception on January 19 includes Nobel Prize Laureates James Watson and Norman Borlaug as well as such prominent individuals as Bruce Ames (winner of the 1998 US President’s National Medal of Science), Gurdev Khush (winner of the World Food Prize), and Ingo Potrykus



(developer of the new "Golden Rice" variety). By signing the petition, members of *AgBioWorld.org* profess their belief that biotechnological advances in plant science "can and should be used to increase crop yields, grow more nutritious plants and reduce dependence on chemicals in order to alleviate hunger and to help preserve the environment."

In addition to hosting the Declaration, the Web site is quickly becoming a repository of essays by and interviews with Professor Prakash that extol the promise agricultural biotechnology holds for the developing world, and challenge arguments often cited by opponents of biotechnology. The site also offers a growing collection of informative, well-reasoned essays on relevant topics authored by Norman Borlaug, Jimmy Carter, Florence Wambugu, US Senator Christopher Bond, Peggy G. Lemaux, The Church of England, the UN Food and Agriculture Organization, and many others. One can also sign up for participation in a listbot service that periodically distributes, via email, articles concerning agricultural technology and its effects on the developing world.

Visitors to this site are invited to join the AgBioView Discussion List to read and post messages to all subscribers of the service. By joining this active global discussion group, one has access to the opinions, suggestions, writings, and queries of a vast array of scientists, physicians, students, and professors world wide, all acutely involved in examining the merits and fallacies pervading the GM food debate. For example, discussions in recent weeks have dealt such varied topic threads as "Pathogenicity and Weediness," "Atomic GM," "Philosophical Underpinnings of Environmentalist Movement," "Luddites and the Modern World," "The Greenpeace 'Peer-Reviewed Study,'" "An Open Letter to Protestors," and "Advice to US Scientists from a British Scientist."

By expressing their support for agricultural biotechnology through the Declaration, scientists hope to provide assurances to the public, journalists, and policy makers of the safety of agricultural biotechnology and its relevance to sustainable development. The Declaration likewise urges policy makers to "use sound scientific principles in the regulation of products produced with recombinant DNA." Certainly, the unanimous call for ongoing, intelligent dialog on this controversial and vital topic has been met in part by the combined efforts of Professor Prakash and the many scientists who share his views.

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UPCOMING MEETINGS

More meetings can be found at: <http://www.isb.vt.edu>

IMPACT OF MOLECULAR BIOLOGY ON CROP PRODUCTION AND CROP PROTECTION

August 20-24, 2000
Minneapolis, Minnesota

The proliferation of mapping, genotyping, and diagnostic methodologies has rapidly expanded the analytical tools available to crop scientists for the analysis of plant genomes. This conference is geared both towards researchers working in the technical developments driving this field and those researchers utilizing new applications.

Session topics include Role of Biotechnology in Agriculture, Structural Genomics of Crops and Pathogens, Functional Genomics of Crops and Pathogens, Transgenic Defense Traits and, Transgenic Enhancement Soil Microbiology. Coverage will include all agronomic crops and their pathogens including: Cereal grains, oil seeds, fruits, vegetables, forage, fiber, and forestry.

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<http://www.healthtech.com/conference/00crp/index.htm>



INTERNATIONAL CONFERENCE ON BIOTECHNOLOGY IN THE GLOBAL ECONOMY: SCIENCE AND THE PRECAUTIONARY PRINCIPLE

September 22 - 23, 2000
Harvard University, Boston

The aim of the Conference is to explore the policy and practical implications of the use of the precautionary principle in the field of biotechnology. The Conference will cover: (a) theoretical, historical and cultural aspects of the principle; (b) previous applications in international environmental and trade law (c) the implications of various definitions for the principle's use in international discussions and negotiations; and (d) social, economic and political implications of the principle in developed and developing countries.

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