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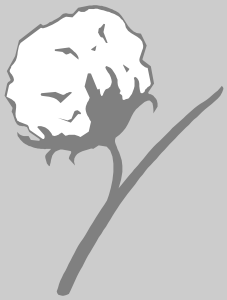

NEWS AND NOTES

"HARVEST OF FEAR"

The Public Broadcasting Service (PBS) programs "Frontline" and "NOVA" combined to present a special report titled "Harvest of Fear," which explores "the intensifying debate over genetically-modified (gm) food crops. Interviewing scientists, farmers, biotech and food industry representatives, government regulators, and critics of biotechnology, this two-hour report presents both sides of the debate, exploring the risks and benefits, the hopes and fears, of this new technology." A video tape and transcript of the program are available from the PBS Web site accessible at <http://www.pbs.org/wgbh/harvest/etc/tapes.html>.

INSECT RESEARCH

TRANSGENIC INSECT RELEASE 2001

Pink Bollworm (PBW), *Pectinophora gossypiella* (Saunders), first came to North America probably in Egyptian seed cotton sent to Mexico, and by 1965 it spread to become a major cotton pest. PBW now infests cotton growing areas of Arizona, New Mexico, Oklahoma, Texas, and parts of Arkansas, California, Louisiana, Mississippi, Missouri, Tennessee, and infests wild cotton in Florida. The National Cotton Council of America estimates that PBW costs the US more than $24 million annually and may be the most destructive pest of cotton world-wide. Cotton crop losses of more than 20% have been reported in Brazil and China. PBW larvae damage cotton when they feed inside the growing cotton boll. Though cotton is the larvae's preferred host plant, it will feed on okra and hibiscus, including kenaf. There is thus a strict USDA APHIS quarantine in infested areas and a concerted effort is ongoing to keep PBW out of uninfested areas like the San Joaquin Valley of California.

PBWs develop from eggs into larvae, pupae, and then adults. Females lay 100–200 eggs on young cotton bolls. The eggs hatch five days later, and the larvae burrow into the boll to feed for 10–14 days. The small, dark-headed caterpillar larvae range from 7–10 mm. Mature larvae either remain in the cotton seed or bore out of the boll, drop to the ground, and pupate in soil crevices. PBW can remain as pupae for as little 8–10 days, or can wait as long as two years before emergence. Adults are nondescript grayish brown moths with a wingspan of 15–20 mm and an average weight of about 17 mg. After emerging from pupae, the female moths mature for a few days, mate, and then start laying eggs one to

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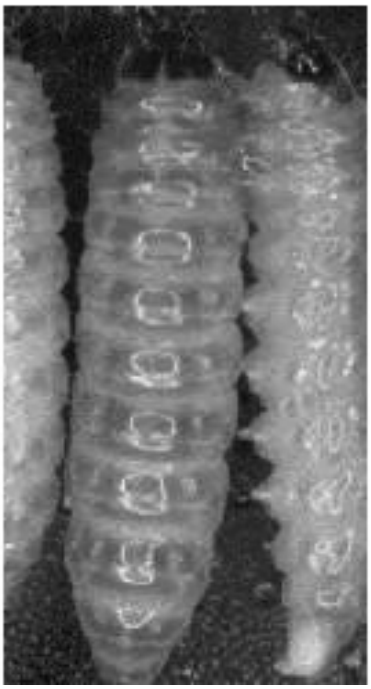
three days later. During long growing seasons, PBW can produce five to six generations. Egg-to-adult development times can be as rapid as 26–32 days. Adults are nocturnal, spend their days hiding, and live about 10 days. Larvae in cotton seed can survive the cotton ginning process, so, unless appropriate measures are taken, live PBW can emerge from stored seed the next spring.



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Sterile Insect Technique Program for PBW Control

A major aim of PBW control has been to protect the San Joaquin Valley of California, the last cotton growing area in the US Southwest that is not generally infested with PBW. The Sterile-Insect Technique (SIT) program, credited with maintaining this pest-free status, was established in 1968 by the USDA, California Department of Food and Agriculture, and the California Cotton Growers.[1,2] PBW are raised in the Pink Bollworm Rearing Facility (PBWRF) in Phoenix, Arizona and sterilized by irradiation before release. Sterile moths are shipped to growing areas and released from May to mid-October when PBW are most active. A ratio of 60 sterile moths to one wild moth is needed to achieve control, so presently SIT is not economically feasible in heavily infested areas.

A long-standing issue in the PBW-SIT program has been the origin of monitoring catches. When SIT managers decide where to spend their valuable sterile insects, they want to put them where there are wild insects, not where there are already lots of sterile ones; therefore, they use traps to catch the insects in the field to assess their numbers. However, they can't easily tell if the insects they catch are steriles from the release, fertile pests from the field, the offspring of matings between "steriles" that somehow escaped sterilization, or between pests and non-sterile "steriles." The development of the genetically-marked mass-reared PBW will therefore provide advantages to SIT program managers in their daily insect deployment strategies based on trap finds.[3]

Development of a Genetically Modified Pink Bollworm

Transgenesis is the modification of phenotype by introduction of (usually recombinant) genes. Marker genes are introduced into the host so that the transformed animals can be selected. An ideal marker gene is distinctive and non-destructive. Green Fluorescent Protein (GFP) and derivatives function as dominant and non-destructive markers in a variety of organisms suggesting that Enhanced Green Fluorescent Protein (EGFP) would work in PBW.

We used a transposable element (TE) to introduce DNA into PBW. Transposons or TEs are bits of DNA that can introduce themselves into a genome and can move themselves from one place in the genome to another through the mediation of an enzyme called transposase. Transposons of some form, though hard to find, have been located in all organisms examined. Transposons can be autonomous or non-autonomous. In an autonomous transposon, the transposase is encoded within the transposon DNA and thus the transposon can move on its own because it can code for its own transposase. In a non-autonomous transposable element, no transposase activity is encoded within the element; therefore, added transposase is required for transposon movement. The ideal situation for using transposons for transgenesis is one in which there is a transposon and transposase that will integrate into the host genome, but are not present in that genome, nor are there any transposable elements similar enough to the putative transforming transposon to interact with it.

Like all organisms that have been examined closely, PBW has DNA sequences related to at least one transposable element,[4] but lacks any TE that interacts with the *piggyBac* TE.[5] The *piggyBac* element was discovered in a moth (*Trichoplusia ni*) cell culture. Because of *piggyBac*'s ability to transpose in a wide range of hosts, it may represent the closest thing yet to a universally applicable transposable element; hence, its ever-increasing use in transformation work.

We made non-autonomous DNA using *piggyBac* for EGFP gene insertion into PBW, and injected PBW embryos (eggs) with a cocktail of two DNA plasmids. The donor plasmid had the EGFP gene flanked by the ends of the *piggyBac* transposon, which are absolutely essential for the *piggyBac* transposase to recognize and integrate a given sequence of DNA into the genome. The second plasmid in the cocktail, the helper plasmid, was DNA that coded for the transposase but lacked one essential end of the transposon necessary for the transposase to work. The *piggyBac* transposase was produced from the helper plasmid when the cocktail was injected, and acted solely upon the donor plasmid because only it had DNA flanked by the two *piggyBac* ends. Since DNA from the donor plasmid did not carry the gene for *piggyBac* transposase, it couldn't move on its own, and since there weren't any such transposases already in PBW,[5] the EGFP marker gene from the donor plasmid became essentially just another gene—albeit introduced by recombinant processes—in the PBW genome. We selected the green transformed PBWs that were progeny of matings of injected insects to start several transformed strains, and transferred one of these strains, 35, to the PBWRF where we compared its biology with the parental strain. Strain 35 is now being expanded for use in the first field trials of a transgenic pest insect.

Laboratory work shows that the EGFP transgene is stable in strain 35 over 20 generations. Additionally, EGFP confers no competitive advantage to the recipient.[6] Transformation did not result in significant changes in the development of the transgenic GFP strain when compared to its non-transformed ancestors, the APHIS strain of PBW. We did see a reduction in egg production and survivability, though not to levels that would hamper the use of this strain in an SIT colony.

This marked transgenic strain of PBW will also allow us to evaluate transgenic insects used in pest control. We want to know more about specific traits (behavior and fitness etc.) of the EGFP transgenic insects under field conditions before we can confidently advance the technology. The first set of trials will be contained in mesh covered field cages in isolated Arizona cotton fields from which insects will be unable to escape. Though the authors anticipate no environmental problems from these marked insects at anytime in the future, they believe these cage studies will provide needed substantiation of the absence of environmental concerns.

Three initial tests are planned with insects that will be radiation sterilized and have their wings clipped to further prevent escape. We want to 1) compare male response to pheromone traps of GFP strain and non-GFP strain PBW; 2) compare male longevity in the field of GFP and non-GFP strain PBW; and 3) compare the abilities of the GFP and non-GFP females to solicit and mate with their male counterparts.

The Future of Insect Control and Reduction of Pesticide Use

Just marking these insects is not our ultimate goal. Even a slight increase in the quality of a sterile insect can translate to substantial gains in the effectiveness of an SIT program. Therefore, we are engineering colony insects to be fertile in the lab but "sterile" when released into the field so they won't have to be irradiated. (See "Is RIDL the Answer?" by Luke Alphey, *ISB News Report*, May 2000, <http://www.isb.vt.edu/articles/may0006.htm>.) Results from our field study this summer will help us in this effort.

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Reexamination of Pest Resistance Management Models Advised

Biotechnology applications to agriculture, if properly integrated into production systems, offer more sustainable and ecologically friendly tools for increasing productivity. Insertion of a gene from *Bacillus thuringiensis* that produces a protein toxic to certain insect pests (Lepidoptera, Coleoptera, and Diptera families) is one such application. Within the Bt organism alone there are over 50 genes with known insecticidal properties. Several of these genes could be deployed simultaneously (if the modes of action are different) to increase crop protection levels and reduce the likelihood that insect populations will become resistant to the Bt toxin.

Because Bt toxins are highly specific to target insects, Bt crops, if deployed cautiously, offer ecological benefits over conventional broad-spectrum insecticides such as the pyrethroids. The toxin produced by the *Cry1Ac* gene is particularly selective for major lepidopteran pests. However, the long-term impact of Bt crops can only be sustained if effective deployment strategies are adopted that hinder the evolution of insects resistant to Bt toxin. Such deployment strategies must be sustainable and remain viable in the event that insect resistance develops. A high dose approach (high gene expression) used in combination with structured refuge areas is the most widely adopted strategy to manage insect resistance, but it requires frequent monitoring for detection of possible insect resistance.

Most strategies for delaying pest resistance are based on theoretical models which assume that the genes conferring resistance are rare. However, work carried out by a team of scientists headed by Bruce Tabashnik at the University of Arizona suggests that this and other assumptions of resistance management models have to be reexamined. The team collected pink bollworm larvae (*Pectinophora gossypiella*) from 300 to 2,000 cotton bolls from 10 cotton fields in Arizona in 1997 and 1998, and from 13 cotton fields in 1999. They found that the frequency of the resistance allele in PBW was not rare, but surprisingly high in 1997; however, the frequency of resistance did not increase from 1997 to 1999 and the Bt cotton remained extremely effective against PBW throughout the examination period.

Two independent methods, a direct and an indirect approach, were used to estimate the frequency of resistance to Bt cotton in field-derived populations of PBW. The direct approach was based on laboratory bioassays of field-derived strains of larvae for susceptibility to Cry1Ac toxin; the indirect approach calculated the relative abundance of live PBWs in field-collected bolls of Bt and non-Bt cotton. With three exceptions, PBW larvae were too scarce in Bt cotton fields to obtain the required numbers to initiate strains. Consequently, at least 100 individuals were collected from non-Bt cotton fields directly adjacent to Bt cotton fields, and forty-one, nine, and 24 individuals were collected from three Bt cotton fields to initiate field-derived strains for analysis. The larvae were reared on wheat germ diet without Cry1Ac, except when the larvae were tested for susceptibility to Cry1Ac in laboratory bioassays.

One or more generations of each field-derived strain were bioassayed on wheat germ diet containing either 0 (control), 3.2, or 10 µg of Cry1Ac per ml of diet to determine their susceptibility. After 21 days, live fourth instar larvae and pupae were scored as survivors. These survivors were then pooled and selected with Cry1Ac over seven generations to create the resistant strain, which ultimately survived 100 µg of Cry1Ac per ml of diet in the final (third) selection.

Using reciprocal crosses between resistant and susceptible

strains, the researchers inferred that survivors from 10 field-derived strains from 1997 were homozygous for an autosomal recessive resistance allele with an allele frequency of 0.16. Though all the 10 strains derived from 1997 had survivors at 10 µg Cry1Ac, only one Arizona strain showed survival at that level in 1998, with an estimated resistance allele frequency of 0.0070. Likewise, in 1999, none of the neonates from 13 strains (over 6,799 individuals) survived exposure to 10 µg of Cry1Ac per ml of diet, a result which yielded an estimated resistant allele frequency of 0. Indirect estimates, based on relative abundance of PBW larvae in Bt and non-Bt cotton bolls, also confirmed that the resistance allele frequency, calculated at 0.13 for 1997, 0.050 for 1998, and 0.11 for 1999, did not increase from 1997 to 1999. These results overlap with the direct estimates for 1997 and 1998, indicating agreement for the two approaches.

The results of the experiments revealed that resistant PBW larvae capable of surviving on Bt cotton were not rare in some Arizona cotton fields in 1997. The estimates of mean resistance allele frequency for 1997 were almost 100 times higher than resistance genes for other lepidopteran pests that attack Bt crops. However, examination of 140,950 bolls of Bt cotton and non-Bt cotton showed that Bt cotton remained effective against lepidopteran pests, contrary to models that predicted an increase in resistance allele frequency. The authors suggested factors that could contribute to the delay of resistant PBW evolution in the field, including the existence of refuges, a reduced rate of development of larvae in Bt cotton fields, and fitness costs associated with resistance.

Source
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Fate and Effects of the Insecticidal Toxins from *Bacillus thuringiensis* in Soil

The incorporation into plants of genes from *Bacillus thuringiensis* (*Bt*) that code for the production of insecticidal toxins (Cry proteins) reduces many problems associated with the use of chemical pesticides, as the toxins are produced continuously within these plants. Impressive control of *Bt*-susceptible insect pests has been obtained with such plants in the laboratory and in the field. However, there is concern that genetically engineered crops may pose risks to natural and agricultural ecosystems.[1] If production exceeds consumption and inactivation by insect larvae, degradation by the microbiota, and abiotic inactivation, the toxins could accumulate in the environment to concentrations that may: 1) constitute a hazard to nontarget organisms, such as the soil microbiota, beneficial insects (e.g., pollinators, predators, and parasites of insect pests), and other animal classes; 2) result in the selection and enrichment of toxin-resistant target insects; and 3) enhance the control of target insects. Accumulation is enhanced when the toxins are bound on surface-active particles in the environment (e.g., clays and humic substances) and, thereby, are rendered less accessible for microbial degradation but still retain their toxic activity.

The toxins produced by *B. thuringiensis* subsp. *kurstaki* (*Btk*; 66 kDa; active against Lepidoptera) and subsp. *tenebrionis* (*Btt*; 68 kDa; active against Coleoptera) adsorbed rapidly (in <30 min, the shortest time studied) on mined clay minerals [montmorillonite (M) and kaolinite (K)], on the clay-size fraction of soil, on humic acids, and on clay-humic acid complexes.[1-2] Only about 10 and 30% of the toxin from *Btk* or *Btt*, respectively, adsorbed at equilibrium, was desorbed by one or two washes with water, and additional washing desorbed no more toxins, indicating that the toxins were tightly bound on the clays. Interaction of the toxins with the clays did not alter significantly the structure of the toxins, as indicated by ELISA examination of the equilibrium supernatants and desorption washes and by Fourier-transform infrared analyses and insect bioassays of the bound toxins. The toxins only partially intercalated M, as determined by X-ray diffraction analyses, and there was no intercalation of K, a nonexpanding clay mineral.

The toxin from *Btk* or *Btt* bound on M, K, or the clay-size fraction was larvicidal to the tobacco hornworm (*Manduca sexta*) or the Colorado potato beetle (*Leptinotarsa decemlineata*), respectively. When free toxin from *Btk* was added to nonsterile soils, larvicidal activity was detected after 234 days, the longest time evaluated.[2]

The binding of the toxins from *Btk* and *Btt* on clays reduced their availability to microbes, which is probably responsible for their persistence in soil. The free toxins were readily utilized, both *in vitro* and in soil, as sources of carbon and/or nitrogen by pure and mixed cultures of microbes, including soil suspensions, whereas the bound toxins were not utilized as a source of carbon, and utilized slightly as a source of nitrogen, but they did not support growth in the absence of exogenous sources of both available carbon and nitrogen. The toxins, free or bound, had no effect on the growth *in vitro* of a spectrum of bacteria (both gram-positive and gram-negative), fungi (both yeast and filamentous forms), and algae (primarily green and diatoms).[1]

The toxin was released in root exudates from Bt corn (NK4640Bt) grown in sterile hydroponic culture and in sterile and nonsterile soil in a plant-growth room.[3-4] Bt corn is maize (*Zea mays* L.) that has been genetically modified to express the *cry1Ab* gene from *B. thuringiensis* to kill lepidopteran pests, especially the European corn borer (*Ostrinia nubilalis*), a major pest in Europe and North America that can reduce yields of corn by 3 to 7% borer $^{-1}$ plant $^{-1}$. The presence of the toxin was indicated by a major band migrating on SDS-PAGE to a position corresponding to a molecular mass (M_r) of 66 kDa, the same as that of the Cry1Ab protein, and was confirmed by immunological and larvicidal assays. After 25 days, when the hydroponic culture was no longer sterile, the band at 66 kDa was not detected (there were several new protein bands of smaller M_r) and the immunological and larvicidal assays were negative, indicating that microbial proteases had hydrolyzed the toxin. By contrast, the toxin was detected after 25 days in both sterile and nonsterile soil, indicating that the released toxin was bound on surface-active particles in rhizosphere soil, which protected the toxin from hydrolysis, similar to results observed with purified toxins.

To estimate the importance of the clay mineralogy and other physicochemical characteristics, which influence the activity and ecology of microbes in soil, on the persistence of the toxin released in root exudates from Bt corn, studies were done in a sandy loam soil amended with various concentrations (3–12%) of M or K in a plant-growth room. Rhizosphere soil from plants of Bt corn were positive 10–40 days after germination for the presence of the toxin when assayed immunologically with Lateral Flow Quickstix.[4] No toxin was detected in any soil with plants of non-*Bt* corn or without plants. All samples of soil in which Bt corn was grown were toxic to the larvae of *M. sexta*, with mortality ranging from 25–100% on day 10 and increasing to 88–100% on day 40, whereas there was no mortality with any soil from non-Bt plants and with soil without plants. In addition, the size and weight of surviving larvae exposed to soils from Bt corn were significantly lower (*ca*. 50–92% lower) than those exposed to soil from non-Bt corn or to soil without plants. The larvicidal activity was generally higher in soil amended with M than with K, probably because M, a swelling 2:1, Si:Al, clay mineral with a significantly higher cation-exchange capacity and specific surface area than K, a nonswelling 1:1, Si:Al, clay, bound more toxin in the root exudates than did K, as has been observed with pure toxin. Nevertheless, mortality in the M and K soils was essentially the same after 40 days, indicating that over a longer time, the persistence of larvicidal activity is independent of the clay mineralogy and other physicochemical characteristics of the soils. The increase in larvicidal activity between 10 and 40 days indicated that the toxin in the root exudates was concentrated when adsorbed on surface-active components of the soils.

The toxin was also released in root exudates of field-grown Bt corn plants, and it remained larvicidal for months after their death and subsequent frost.[4] Although the larval mortality in rhizosphere soil from plants of field-grown Bt corn ranged from 38–100% and the coefficients of variation were large, the size and weight of the surviving larvae were reduced by 40–50% when compared with soil from non-Bt corn or from soil without plants.

To determine whether the release of the Cry1Ab protein is a common phenomenon with transgenic Bt corn, the release of the Cry1Ab protein in the exudates of 13 Bt corn hybrids, representing three different transformation events (Bt11, MON810, and 176), and of their isogenic nontransgenic counterparts was studied. All samples of rhizosphere soil from the 13 hybrids, whether grown in the plant-growth room for 40 days or in the field (harvested after the production of ears of corn), were positive for the presence of the toxin when assayed immunologically, and all samples were toxic to the larvae of *M. sexta*, with mortality ranging from 38–100%. No toxin was detected immunologically or by larvicidal assay in any soil in which plants of non-Bt corn or no plants had been grown. In addition, the weight of surviving larvae exposed to soils from Bt corn was significantly lower (80–90%) than those exposed to soils from non-Bt corn or without plants.

These results indicated that the release of the toxin in exudates from roots of Bt corn is a common phenomenon and that the released toxin could accumulate in soil and retain insecticidal activity, especially when the toxin is bound on surface-active soil particles and, thus, becomes resistant to degradation by microorganisms. Although some

toxin was probably released from sloughed and damaged root cells, the major portion was derived from exudates, as there was no discernable root debris after centrifugation of the Hoagland's solution when plants were grown for 25 days in hydroponic culture.

In addition to the large amount of toxin that will be introduced to soil in plant biomass after harvest and some that will be introduced in pollen released during tasseling, these results indicated that the toxin will also be released to soil from roots during the entire growth of a Bt corn crop. The presence of the toxin in soil could improve the control of insect pests, or the persistence of the toxin in soil could enhance the selection of toxin-resistant target insects and constitute a hazard to nontarget organisms, as receptors for the toxins are present in both target and nontarget insects. Consequently, nontarget insects and organisms in higher, and perhaps also in lower trophic levels, could be susceptible to the toxins.

To determine the effects of the Cry1Ab toxin released in root exudates and from biomass of Bt corn on various organisms in soil, 20 medium-size earthworms (*Lumbricus terrestris*) were introduced into soil planted with Bt or non-Bt corn or amended with 1% biomass of Bt or non-Bt corn and soil not planted or amended. After incubation, the numbers of earthworms were counted and their weight determined. Representative worms were dissected, and soil from the guts, as well as from the casts, was analyzed for the presence of the Cry1Ab protein by immunological and larvicidal assays.[5]

There were no significant differences in the percent mortality and weight of earthworms after 40 days in soil planted with Bt or non-Bt corn or not planted or after 45 days in soil amended with biomass of Bt or non-Bt corn or not amended. However, the toxin was present in both the casts and guts of worms in soil planted with Bt corn or amended with biomass of Bt corn, whereas it was absent in casts and guts of worms in soil planted with non-Bt corn or not planted and in soil amended with biomass of non-Bt corn or not amended. When worms from soil with Bt corn or amended with biomass of Bt corn were transferred to fresh soil, the toxin was cleared from the guts in one to two days. All samples of soil amended with biomass of Bt corn and from the rhizosphere of Bt corn were positive for the presence of the toxin and were lethal to the larvae of *M. sexta* after 45 and 40 days, respectively, whereas there was no mortality in soil amended with biomass of non-Bt corn, in rhizosphere soil of non-Bt corn, or in soil with no plants or not amended, which were also negative in the immunological assays. There were no statistically significant differences ($P>0.5$) in the total numbers of nematodes and culturable protozoa, bacteria (including actinomycetes), and fungi between rhizosphere soil of Bt and non-Bt corn or between soils amended with Bt or non-Bt biomass.

These results suggest that despite its persistence in soil, the toxin released in root exudates of Bt corn or from the degradation of the biomass of Bt corn is not toxic to a variety of organisms in soil. The toxin was detected in the guts and casts of earthworms grown with Bt corn and in soil amended with biomass of Bt corn, indicating again that the released toxin bound on surface-active particles in soil, which protected the toxin from biodegradation, similar to what has been observed with purified toxins. Because only one species of earthworms and only total culturable microorganisms and nematodes were evaluated, more detailed studies, including studies using techniques of molecular biology, on the composition and diversity of these groups of organisms are necessary to confirm the absence of effects of the Cry1Ab toxin on biodiversity in soil.

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PLANT RESEARCH

ARABIDOPSIS RESEARCH BEARS FRUIT IN ORANGE TREES

Tree breeding programs traditionally have been hampered by the long generation times of many tree crops. New findings reported by Martinez-Zapater et al. in the March issue of *Nature Biotechnology* indicate that, at least for citrus species, the generation time from seed to seed can be slashed from six years to as little as one or two by the introduction and overexpression of either one of two *Arabidopsis* floral identity genes, *LEAFY* or *APETALA1*.[1]

Many perennial species, including most commercial fruit and nut tree species, exhibit a juvenile phase characterized by a lack of an ability to flower and reproduce. This juvenile phase is sometimes distinguished by a markedly different leaf shape and overall growth morphology. In English ivy (*Hedera helix*), the juvenile form is the familiar trailing vine with five-pointed lobed leaves, whereas the adult form is upright and shrub-like, bearing smooth, obvate leaves and floral clusters. In citrus species, the juvenile phase can last between six and 20 years, depending on the species, with another two years often required before the full productivity of a new cross can be assessed. The practical impact of this juvenile phase is that any breeding program for citrus often requires the investment of decades of work before any real results are achieved.

Martinez-Zapater and collaborators attempted to get around this problem by overexpressing two *Arabidopsis* genes, *LEAFY* (*LFY*) and *APETALA1* (*AP1*), previously shown to be capable of inducing early flowering when ectopically expressed. *LFY* and *AP1* are part of a group of master regulatory genes that are believed to control floral meristem identity. Homologs of both genes have been identified in a number of plant species leading researchers to speculate that the function and action of these genes may be conserved across species. In fact, when both genes were overexpressed individually from the powerful, constitutive 35S promoter in a wildtype *Arabidopsis* plants, researchers found that the resulting transgenic plants exhibited precocious flowering, with all axillary buds converted to terminal flowers.[2,3]

Initial attempts to capitalize on this phenotype have yielded mixed results. Encouragingly, overexpression of the *LFY* gene (either the cDNA of the *Arabidopsis* gene or the endogenous homolog) in *Populus* lead to early flowering in regenerated plants, with flowers appearing on the plants within a year of transformation.[3,4] However, both the timing of flowering and the shape of the flowers in these plants were aberrant, with the formation of flowers coinciding with the production of leaves, something never seen in wild type trees, and the termination of both the primary and secondary meristems in terminal flowers.

Martinez-Zapater and collaborators speculate that the aberrant phenotype caused by the overexpression of *LFY* may be due to the gene's fairly central regulatory role leading to its involvement in multiple developmental pathways. Studies examining the overexpression of *LFY* and *AP1* in *ap1* and *lfy* mutant backgrounds, respectively, indicate that the *AP1* gene product functions downstream of *LFY*. For this reason, it was hoped that transformation of plants with the *AP1* gene would still produce early flowering, but with fewer undesirable side effects.

To study the effects of the overexpression of those genes in citrus, Martinez-Zapater and collaborators used *Agrobacterium*-mediated transformation to introduce constructs containing cDNA clones for the *Arabidopsis LFY* or *AP1* genes linked to the 35S promoter, into citrange, a hybrid between *Citrus sinesis* L. Osbeck and *Poncirus trifoliata* L. Raf. Transformation yielded 22 transgenic lines carrying the *35S:LFY* construct and 12 lines carrying the *35S:AP1* construct. When compared to control plants, six of the *35S:LFY* plants and two of the *35S:AP1* plants exhibited precocious flowering, producing flowers in two to 20 months following transformation, instead of the usual five or six years observed for control plants. Interestingly, all of the *35S:LFY* plants and eight of the *35S:AP1* plants exhibited other signs of adult morphology in the form of reduced production of thorns and/or increased branching. Plants from both sets of transgenic lines produced normal, fertile flowers that developed into normal fruit.

Flowers on the transgenic plants were either allowed to self-fertilize, or pollen was collected from them and used to fertilize flowers of another variety of citrus, mandarin. The early flowering phenotype was transferred to both types of progeny, yielding plants that produced flowers within the first year following germination.

Despite the significant number of early flowering plants produced by transformation with the *35S:LFY* construct, there were serious developmental problems associated with these lines. *35S:LFY* plants were slow growing and weedy, displaying a weeping growth habit and severely curled leaves. In addition, nine of the lines aborted the primary meristem, only resuming growth after a significant period

of time from axillary buds. These problems correlate with the aberrant growth habits observed in the *Populus* species ectopically expressing *LFY*, emphasizing the global role of this regulatory gene.

Plants transformed with the 35S:*AP1* construct exhibited largely normal growth, beyond the early flowering phenotype and reduced juvenile features. Interestingly, both the original transformants and their progeny produced flowers in the spring, the normal flowering time for citrange. This appears to indicate that the *Arabidopsis AP1* gene product is interacting with endogenous regulatory elements in these plants, allowing the plants to respond to environmental signals and produce a normal flowering response.

Tantalizingly, the authors propose a unique way to use these plants to quickly assess the value of a new cross, without creating a transgenic end product. By using a stably transformed *35S:AP1* plant as one parent in each cross, 50% of the progeny would contain the transgene, producing flowers and fruit within one to two years of germination. Using inducible recombination, the transgene could then be excised and nontransgenic plants regenerated from selected shoots.

The precise function of *AP1* is still unknown even in *Arabidopsis*. Despite the promise of these early results, more detailed studies are required to determine if there are any deleterious side effects associated with the overexpression of this gene that have yet to be detected. However, with the shortened flowering time of these plants, at least the time required to perform such studies, should be significantly reduced.

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GNA Expressing Potatoes

The insertion of specific lectin genes into crops is showing value in IPM programs for controlling pestilent organisms. Lectins belong to a broad group of bioactive peptides called defensins that elicit a variety of responses that can defend plants from a range of factors including environmental stress, predation by insects, and infestations by bacteria, fungi, and nematodes. Many agricultural plants lack lectin defenses, however, making them susceptible to yield losses from pest infestations.

John and Angharad Gatehouse of Durham University, UK, and their research team report successfully inserting lectin genes into potatoes to facilitate the biological control of lepidopteran larvae.[1] Their goal was to facilitate biological control of the plant-feeding larval form of *Lacanobia oleracea*, tomato moth, using both the lectin-expressing transgenic potato and a parasitoid wasp, *Eulophus pennicornis*. In effect, they succeeded in producing a pesticidal potato that specifically harms the phytophagus moth larvae without impairing the beneficial wasps that prey on the pest.

GNA (Galanthus nivalis agglutinin) is a naturally-occurring lectin found in snowdrop plants (*Galanthus nivalis*). The amaryllis, iris, and lily families are noted for strong plant defense mechanisms that include defensin production. Lectins are favored over other pesticidal plant compounds because of their low toxicity to humans and domesticated animals at levels that are effective against insect larvae, and GNA does not appear to damage other organisms once the pest ingests it.

In this study, the Gatehouse team used potato plants expressing GNA at levels equaling 1% of the total soluble proteins of the plant. These levels had been shown to effectively reduce *Lacanobia oleracea* damage in previous research trials.[2] The transgenic plants were compared to control plants without the GNA gene. Greenhouse trials were conducted on 18 transformed and 18 non-transformed potatoes plants. Each plant was manually infested with 15 third-instar caterpillars. Ten days later, a first wave of parasitoid wasps were released into one of the greenhouses. Another set of wasps was introduced two days later just as the caterpillars were reaching the fifth instar stage. Data was collected seven days after the initial introduction of wasps.

Tomato moth caterpillars damaged significantly more leaves per plant on the control potatoes in both greenhouse trials. This result supports previous studies indicating the

effectiveness of GNA on reducing *Lacanobia oleracea* predation. Non-transgenic plants infested with the larvae that were exposed to the parasitic wasps showed less leaf damage than those not exposed to wasps; however, leaf damage was most reduced in lectin-expressing transgenic plants subject to larvae predation by wasps.

GNA-expressing plants did not significantly decrease the population of *Lacanobia oleracea* larvae. However, the GNA did affect a significant decrease in caterpillar growth, thereby causing the reduction in plant feeding damage. The parasitoid wasps caused significant decreases in caterpillar number and weight on both the control and GNA-expressing plants. GNA did not enhance the susceptibility of the caterpillars to wasp predation.

The study evaluated whether GNA exposure harmed the parasitoid wasps. Analyses indicated no significant impact on wasp development. A slight reduction in wasp number for the GNA trials was not statistically significant and did not reduce the ability of the wasps to parasitize the caterpillars.

This study demonstrated that GNA plants, used in conjunction with biological control, significantly reduced crop damage to a greater extent than either strategy used alone. The advantages of using this system are that it is less likely than traditional pesticide treatment to produce resistant pests, is less toxic to the beneficial parasitic *Eulophus pennicornis*, and thus would likely reduce the need for environmentally harmful and costly pesticides.

Sources
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Upcoming Meetings

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

10th European Congress on Biotechnology

Biotechnological Challenges in the New Millennium
July 8–11, 2001
Palacio de Congresos, Madrid, Spain

The conference, organized by the Spanish Society of Biotechnology (SEBIOT), will convene specialists from academia and industry to cover all fields of knowledge and application relating to biotechnology in Europe. The Congress will consist of over 60 symposia to cover research and application of biotechnology on topics to include the following:

- Biotechnology For Animal And Human Health
- Applied Genome Research
- Microbial Biotechnology
- Agrobiotechnology
- Environmental Biotechnology
- Biochemical Engineering
- Biocatalysis
- Biosafety
- Biotechnology And Society

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Current Issues in Food Biotechnology

Second Joint Symposium on Food Safety and Nutrition
July 11–13, 2001
Adelphi, Maryland

Held at the University of Maryland University College, the subject for this symposium, "Current Issues in Food Biotechnology," has been selected to enable those involved in different aspects of the application of the methods of modern biotechnology to the production and processing of foods to meet and discuss current issues in this important area. The program will consist of invited lectures and discussion sessions. Topics to be covered may include, but are not limited to, international perspectives; future pros-

pects (what's in the pipeline, applied nutrition); safety (allergenicity, testing, environmental); detection and analysis; communication of issues associated with GMOs; and consumer views. Agenda items include addresses on the following topics:

- StarLink - A case study
- International perspectives
- Safety considerations on allergens
- Gene transfer
- Current studies on safety
- Governmental agency responsible for validating methods and detection
- Options to manage agbiotech in food processing and marketing
- Future prospects
- New technologies
- Consumer view
- Industry view

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Biotechnology 2001

Educators' Conference for High School and Community College Teachers
July 26–28, 2001
Blacksburg, Virginia

This conference, organized by the Fralin Biotechnology Center on the Virginia Tech University campus, consists of two mornings of seminars with hands-on workshops in the afternoons and workshops all day on Saturday. Participants will hear speakers on a wide range of subjects, from HIV and the digestive system to transgenic plants for productivity and nutrition. The Keynote Speaker will be Bruce Alberts, President of the National Academy of Sciences.

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Transgenic Animal Research Conference III

September 9–13, 2001
Lake Tahoe, California

This is the third international meeting hosted by University of California, Davis to bring together representatives from the leading laboratories worldwide doing state-of-the-art work on transgenic research in non-murine animals, including livestock, fish, and poultry species. Presentations will address cutting-edge methodology, technical improvements, and current progress towards producing transgenic animals for biomedical and agricultural applications. The intent of these meetings is to bring together scientists to discuss progress, problems, and potential application of transgenic technology for animal applications.

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II Brazilian Congress on Biosafety and II Latin American Symposium on Transgenic Products

September 26–28, 2001
Salvador, Bahia, Brazil

This event, organized by ANBio (The National Biosafety Association) and affiliated with ABSA (the American Biological Safety Association), will discuss solutions for the main problems in biosafety, the interrelations between bioethics and biosafety, the legal rights related to the human genome and the new stem-cell therapies, access to genetic resources in the country, the biosafety regulatory framework, and the ethical code for gene manipulation. Labeling of genetically modified foods and public perception of biotechnology will also be among the subjects debated.

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