CHAPTER #

Technological Advances to Enhance Agricultural Pest Management

T.A. Miller,* C. R. Lauzon and D. J. Lampe

Abstract

Biotechnology offers new solutions to existing and future pest problems in agriculture including, for the first time, possible tools to use against insect transmitted pathogens causing plant diseases. Here, we describe the strategy first described as Autocidal Biological Control applied for the development of conditional lethal pink bollworm strains. When these strains are mass-reared, the lethal gene expression is suppressed by a tetracycline repressor element, which is activated by the presence of chlorotetracycline, a normal component of the mass-rearing diet. Once removed from the tetracycline diet, the lethal genes are passed on to offspring when ordinary lab-reared pink bollworms mate with special lethal strains. Lethality is dominant (one copy sufficient for lethality), expressed in the egg stage and affects all eggs (100% lethal expression). The initial investment by the California Cotton Pest Control Board is an outstanding example of research partnerships between agriculture industry, the USDA and land grant universities.

Introduction

The control of pest insect populations by genetic means came into its own during the last century as noted by Davidson.[1] Transgenic insect technology stands to impact agricultural pest management in several areas. The first obvious applications as suggested by Ashburner et al[2] are use of transgenic insects in Sterile Insect Technique programs by improving the genetic control mechanism.

Another application of transgenic technology is the use of female-killing factors in mass-rearing colonies. It was suggested by Heinrich and Scott[3] that releasing only sterile males is much more efficient and cost effective than rearing, sterilizing and releasing both sexes. One way to switch mass-rearing to a males-only production is by applying transgenic methods, which are faster than waiting to find appropriate chance mutations.

Still another opportunity presented by advances in biotechnology is disruption of transmission of plant pathogens by insects. First conceived of in human disease (Chagas disease) protection by Frank Richards and colleagues,[4] paratransgenesis methods are suitable for delivery of anti-disease reagents to a variety of pathogens.

A number of plant disease complexes are potential targets for control. Some of the older and more established plant pathogens and diseases are rice stripe virus, Tristeza virus, Citrus canker, Citrus Greening and Citrus Variegated Chlorosis of citrus, Pierce's disease and Grape Yellows of grapevines, a variety of scorch diseases of ornamentals and crop plants, Curly Top Virus

*Corresponding Author: T.A. Miller—Entomology Department, University of California, Riverside, CA. Email: Thomas.

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transmitted by beet leafhopper and Cotton and tomato leaf crumple and curl diseases caused by viruses transmitted by insects (whiteflies).

There are no cures for any of the diseases mentioned above and treatments include removal of the ailing plants as inoculation sources. The vector insects may be treated with insecticides as an indirect method to prevent the spread of the pathogen, but this can put a burden on nontarget organisms and might disrupt Integrated Pest Management (IPM) schemes. While biotechnology offers hope for crop protection from these and other diseases, the Pew Foundation[5] reported in early 2004 that the regulatory apparatus in the United States lacks experience and procedures for approving the new methods.

The symbiotic control of Chagas disease mentioned above employs recombinant tactics, but Peter Cotty[6] reported a simpler form of symbiotic control in which he selected a strain of *Aspergillus flavus* that did not secrete aflatoxin and used it as a product (AF-36) to treat soil ahead of planting time to competitively displace the *A flavus* responsible for aflatoxin contamination of cotton seed. One treatment of AF-36 reportedly protects one field for several years and natural dispersal protects surrounding fields downwind from the treatment area.

Another symbiotic control method that was granted permits for field trials is protection against dental caries offered by a selected strain of *Streptococcus mutans*.[7] Jeffrey Hillman cofounded a company (Oragenics, Inc)[8] partly to develop *S. mutans* as a treatment against tooth decay. Instead of symbiotic control, this application is called by the developers, replacement therapy.

Other symbiotic control applications include possible treatments for inflamed bowel disease (IBD)[9] and protection against HIV.[10] Thus applications span the field of agriculture and medicine. Whenever major technological breakthroughs occur, opportunities abound.

Sterile Insect Technique

Successful use of genetic control methods was first applied by mass-rearing target insect pests, irradiating to produce sterility and releasing overwhelming numbers daily to drastically reduce chances of mating between members of fertile wild-type populations. The method, known as Sterile Insect Technique (SIT) was developed by Edward Knipling.[11] SIT is available only for the most economically compelling of pest insect complexes with compatible biology because of the high cost of operations. The biology of the target pest must allow mass-rearing, transportation, handling the target population must be in a defined area where migration does not dilute the effectiveness of the sterile release insects.

The SIT operations to control pink bollworm, *Pectinophora gossypiella* (Saunders), in California were established by the Cotton Pest Control Board in 1968.[12] Exposure of pink bollworm pupae to gamma radiation from ^{60}cobalt sources in the SIT program has fitness costs as described by Van Steenwyk et al[13] and Miller et al.[14]

The California Cotton Pest Control Board supported a project in the 1980s to produce sterile insects using the modern ability to make conditional lethal transgenic pink bollworms. As described in Miller,[15] the elements necessary to achieve this goal included finding a transformation protocol, a marker gene to use for selection and a conditional lethal gene designed in such a manner as to allow mass-rearing but capable of passing on dominant lethal genes to any offspring from mating between released insects and wild types. A single copy of the gene (in the heterozygote progeny) must be fully lethal in the egg stage for the strategy to work.

The most difficult part of this process, finding a lethal gene, was actually done first when Carl Fryxell[16] realized that the mutant *Notch* gene he was studying in *Drosophila melanogaster* had conditional lethal properties. The other necessary elements were reported independently about the same time including use of fluorescent marker genes such as green fluorescent protein (GFP) by Doug Prasher[17] and the discovery of the piggyBac element by Mac Fraser.[18]

After all of these separate components were identified, Steve Thibault[15,19] put them together with a specially designed *Bm*A3 actin promoter from *Bombyx mori* to make a plasmid. Upon injection into pink bollworm eggs along with a piggyBac helper plasmid supplied by Al

Handler of the USDA, Steve and John Peloquin achieved transformation on the first try in February of 1998. Luke Alphey[20] found the ideal combination of transcription factors and lethal genes from another *Drosophila* lethal gene candidate that eventually proved to be the winning combination and was used to develop a working strain of pink bollworm with single genes proving 100% lethal in eggs (Greg Simmons, personal communication, 2005).

Symbiosis and Pierce's Disease

The principles of symbiotic control were described by the Frank Richards and colleagues[4] early on. As newer applications are developed, the principles are affirmed and modified to fit each case. The key feature of symbiotic control is that the delivery agent must come from the natural cycle as established in the field. Thus in the Chagas disease application, the microbial symbiont, *Rhodococcus rhodnii*, resides in the hindgut of the vector insect, *Rhodnius prolixus*, and therefore has access to the protozoan pathogen, *Trypanosoma cruzi*, occupying the same space. Moreover, *R. rhodnii* is known to supply nutrients to the vector insect and therefore has developed a symbiotic relationship with the host insect. The host insect vector has natural tendency to selectively retain the symbiont. A coprophagic habit by triatomine insects ensures that feces are sampled on a regular basis repeatedly supplying the symbiont to the hindgut.

The dental caries example given above[7] also picks a microbial symbiont, *Streptococcus mutans*, which occupies and evolved to reside in the oral cavity of humans. Therefore there is a predisposed mutualism between the human and the symbiont. The tooth decay replacement therapy tactic removed from the symbiont by recombinant methods the genes responsible for supplying enzymes to catalyze the conversion of glucose to lactic acid, a major source of tooth decay.

The selection of symbiont in the aflatoxin contamination[6] application also mentioned above found a candidate symbiont in the natural ecosystem. AF-36 is named for the 36th isolate of *Aspergillus flavus* that Peter Cotty screened from agricultural fields in Arizona where the strategy was developed for the Arizona cotton industry.

An application for symbiotic control of Pierce's disease has been described.[21,22] Pierce's disease is caused by a specific strain of the pathogen, a bacterium, *Xylella fastidiosa*, that is transmitted physically by the vector insect, a type of leafhopper called a sharpshooters. The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, a fairly recent arrival to California, is a far more serious threat to spread the pathogen that any of the native sharpshooters. Various strains of *Xylella* are acquired by GWSS or related sharpshooter insects from the act of xylem feeding on plants.

Several symbiotic bacteria are also acquired by GWSS from the xylem fluid of host plants during feeding. Both symbionts and *Xylella* strains form biofilms on the lining of the buccal cavity in the foregut of the insect. From here they detach and are swept into the xylem fluid of the next host plant during ordinary feeding and probing by the vector insects. A strain of *Xylella* becomes pathogenic to a given host plant when it physically clogs the xylem vessels causing "scorch" symptoms. One strain of *Xylella fastidiosa* causes Pierce's disease of grapevines; another strain causes oleander leaf scorch (OLS) in the ornamental oleanders. An unprecedented epidemic of OLS in southern California has destroyed thousands of these plants and threatens other ornamental plants due to the presence of still other strains of the pathogen all being cycled by the extremely active GWSS.

Carol Lauzon[21,22] identified symbiotic bacteria from extractions of the heads of GWSS collected from the endemic infestation area. The common endophyte, *Alcaligenes xylosoxidans* var. *denitrificans* (*Axd*) was found along with a few other bacteria and *Axd* has properties suitable as a delivery vehicle for anti-*Xylella* strategies.

Again, *Axd* must occupy exactly the same niche as the target pathogen. This meant that *Axd* had access to *Xylella fastidiosa* and once introduced into the disease cycle could disrupt the transmission between vector insect and host plants.

Bacterial Transgenesis and the Suppression of Horizontal Gene Transfer

Axd is an attractive candidate bacterial species to deliver anti-*Xylella* factors in either plants or sharpshooter vectors, but very little work has been done on this species with regard to genetics or physiology. Its genome has also not been sequenced which limits the approaches one can take to modifying it genetically.

Fortunately, there are broad host range tools that can be employed to modify *Axd* in a sophisticated way despite our limited knowledge of its genetics. Since modified strains of *Axd* are meant for environmental release, concerns about drug markers and horizontal gene transfer must be incorporated into the design of transgenic *Axd*. We have employed the *Himar1 mariner* transposon carried on a suicide plasmid to introduce transgenes into the chromosome of *Axd*.[23] *Himar1* is a eukaryotic transposon of the *mariner* transposable element family that works very well in phylogenetically diverse organisms, including bacteria and archae.[23,24,25] We reasoned that since this element is not normally found in prokaryotes, the chances of it being mobilized from the *Axd* chromosome in the future are essentially zero. The transgenesis system that we have developed is simple to use, is easily mated into *Axd* from *E. coli* via the broad host range RP4 origin of transfer, and results in chromosomal insertions that are stable and easily isolated due to the transfer of kanamycin resistance (kanR) contained in the transposon to *Axd*. Because of concerns over widespread drug resistance in bacteria, the kanR gene can be removed later using FLP recombinase since the kanR gene is flanked by direct repeats of the recognition site of this enzyme.[26,27] The resulting strains of *Axd* carry no drug markers.

Although chromosomal insertions of DNA are inherently stable, bacteria do undergo lateral DNA transfer and thus concerns remain over the horizontal transfer of novel transgenes from *Axd* to other bacterial species. Indeed, this is one of the chief concerns expressed by regulators when evaluating transgenic bacterial species aimed at environmental release. We recently tested one genetic system in *Axd* that can suppress the transfer of DNA from *Axd* to other bacteria dramatically. This is the *colE3*/ *immE3* system from the plasmid ColE3-CA38 that encodes the antibacterial protein colicin and its immunity factor.[28] Colicin /*immE3* is a kind of toxin/antidote system commonly found on plasmids that helps to ensure plasmid maintenance by the bacterial cells that carry them.[29] In such systems, a long-lived toxin is produced in addition to a short-lived antidote to that toxin. As long as both are produced, the cell is viable. If the cell loses the antidote gene for any reason (carried naturally on the ColE3-CA38 plasmid), the cell will die since the toxin remains behind to kill the cell. Similarly, cells that only receive the toxin gene will die since they do not also receive an antidote. Colicin E3 targets 16S ribosomal DNA, cleaving it near its 3' end, thus interfering with ribosome synthesis. The product of the *immE3* gene blocks this function, allowing the cell to live. Linking transgenes to colicin is an ideal way to prevent the horizontal transfer of the transgenes since recipient cells will be killed by colicin if horizontal transfer were ever to occur.

We tested this system in *Axd* by creating strains that carried the *immE3* gene on the chromosome. To these strains we introduced either plasmid pVLT31 (a broad host range matable plasmid) or pEDF5 (pVLT31 carrying the *colE3* gene that produces colicin).[30] We then attempted to transfer pVLT31 or pEDF5 from *Axd* to *E. coli*. While pVLT31 was easily transferred from *Axd* to *E. coli*, pEDF5 was never recovered. Horizontal transfer of pEDF5 from *Axd* to *E. coli* was thus suppressed by the presence of the *colE3* gene at least by a factor of 3×10^{-7}.

Anti-*Xylella* Factors

Progress on the development of anti-*Xylella* factors has been frustrated by the difficulty in culturing this bacterial species and our limited understanding of how it causes disease. Despite the availability of complete or partial genome sequences for four different *Xylella fastidiosa* strains (Temecula 1, 9a5c, Ann-1, and Dixon), our knowledge of how this bacterium functions is very incomplete.[31] Nevertheless, at least three promising avenues have appeared for anti-*Xylella* factors and more are likely to follow.

The first of these factors are antimicrobial peptides. These peptides are relatively short (<10 kDa) and have been isolated from a wide variety of living organisms where they form the basis of the innate immune system.[32] Most antibacterial peptides are thought to act by disrupting the cell membranes of pathogens leading to cell lysis. Importantly, some of these can have comparatively narrow specificities offering the possibility of isolating peptides that have anti-*Xylella* activity but not affecting the other bacteria that inhabit grape xylem. Several anti-*Xylella* antibacterial peptides have been recently reported.[33]

A second class of anti-*Xylella* factor is likely to be single chain antibodies (scFv's). Single chain antibodies are synthetic genes that unite the antigen binding domains of vertebrate antibody heavy and light chains into a single gene by means of a synthetic linker. These genes can be expressed and secreted from bacteria, and can be created as libraries of billions of different members that can be screened against virtually any antigen. Purified proteins and even entire cells can be used to screen such libraries which can allow the targeting of cell surface factors that are important in the growth and pathogenicity of *Xylella*. Moreover, scFv's can be linked to toxins or antibacterial peptides to deliver them directly and specifically to a particular target.[34]

Finally, factors that can interfere with cell-cell communication have been proposed as anti-*Xylella* factors. *Xylella* is known to form biofilms inside grapevine xylem and in its insect vector. Importantly, this biofilm formation has been implicated in its pathogenicity. *Xylella* biofilms are formed in response to a diffusible alpha, beta unsaturated fatty acid signal molecule. Interference with this signal molecule has been suggested as a means to control *X. fastidiosa*.[35]

Ecological Microbiology

A common and likely most appreciated descriptor of microorganisms in ecosystems is that of governor. Microbes govern many activities, such as material cycles, mediating the movement of organic and inorganic compounds on our planet. In doing so, they modulate pH balance and climate, and regulate fluxes. These activities not only occur on a global scale, as we often describe similar activities in an animal gut. Thus, introduction of a modified autochthonous or allocthonous microbe into the environment tends to elicit concerns by some that the natural order of a system or systems, or at least communities within a system, may be disrupted and result in a dysbiosis.

The use of *Alcaligenes xylosoxidans denitrificans* (*Axd*) in the management or control of PD requires that *Axd* remain in ecosystems for limited but effective periods of time and cause minimal and reversible, or no disruption to a host or ecosystem. To begin to assess efficacy and risk associated with the use of *Axd* in the field, we conducted studies aimed to monitor the fate of *Axd* in soil, water, and plant ecosystems under semi-natural conditions. We also examined the potential of *Axd* to engage in horizontal gene transfer.

To assess the efficacy and risk of use for *Axd* in the field we employed Real Time- Polymerase Chain Reaction (RT-PCR) to semi-quantitative *Axd* growth in lake water under semi-natural conditions. We found that *Axd* grew better in autoclaved lake water than in lake water that contained indigenous microbial populations. Thus, competitive attributes associated with established microbial communities overrode the ability of *Axd* to establish within these communities.

Axd growth was also monitored in soil and on leaf surfaces under semi-natural conditions using microbiological and molecular techniques. *Axd* was not retrieved from soils containing indigenous microbial populations unless the soil was autoclaved. *Axd* was retrieved from leaf surfaces from citrus, strawberry, sage, and basil. We are currently examining the effect of introducing *Axd* to citrus leaf microbial communities using denaturing gradient gel electrophoresis and terminal restriction fragment length polymorphism.

We also initiated studies whereby *Axd* was screened for the presence of endogenous plasmids. Endogenous plasmids have been shown by Taghavi et al[36] to engage in horizontal gene transfer (HGT) to members of endophytic communities in poplar trees. We have found that *Axd* can be introduced and recovered viably from citrus xylem, therefore, we began our assessment

of the propensity of *Axd* to engage in HGT. We first screened *Axd* for the presence of endogenous plasmids. A strain of *E. coli* containing a single copy plasmid was used as a control in our survey. Plasmid preparations were conducted to screen for "very low" copy, "medium-low," and "high copy" plasmids using agarose gels. We also conducted to pulse field gel electrophoresis (FIGE) to visualize plasmids that range in size from 50-200 kb and that would not be detected on standard agarose gels used in our survey. Some smeared material was detected on the FIGE gels near 200 kb size, however, this materials was likely genomic DNA. Thus, our data suggest that *Axd* does not contain any endogenous plasmids up to 150 kb that would be horizontally transferred to other bacteria in nature.

We subsequently examined the likelihood that *Axd* could acquire plasmids in nature by monitoring transfer and uptake of two plasmid vectors, DsRed (pIRES-DsRed Express, Invitrogen) and pTZ18r (Amersham Biotech). Transformation attempts included both chemical and electroporation protocols. *E. coli* was used as a control. In both cases, *Axd* resisted transformation while *E. coli* was successfully transformed.

To create a more natural environment whereby *Axd* may be transformed, we studied HGT potential between *Axd* and strains of *E. coli* and *Shigella* sp. that carry fluorescent and antibiotic-marked endogenous plasmids. In coculture studies, *Axd* did not acquire the plasmids from either *E. coli* or *Shigella* sp. where control organisms were transformed. This study is currently being examined with an *Alcaligenes* sp. that contains a plasmid. It is hypothesized that a related species is likely to transfer genes to *Axd* than a more distant relative.

A complete understanding of the risks associated with the introduction of a modified bacterium into an ecosystem includes knowing if the bacterium engages in quorum sensing, or the behavioral language of bacteria. Community members communicate with their own and other species by releasing and responding to the accumulation of signaling molecules known as auto-inducers in their local environment. These auto-inducers, or signaling molecules are assembled into bacterial messages that represent the response of bacteria sensing other bacteria in their local environment. This phenomenon of cell to cell communication in bacteria is the mechanism behind the monitor and coordinated activities bacteria exhibit in response to cell density. Quorum sensing regulates many bacterial behaviors such as symbiosis, sporulation, conjugation, virulence, antibiotic production, and biofilm formation.[37] Thus, we screened *Axd* for the production of acyl-homoserine lactones, autoinducers of Gram negative bacteria. We found that *Axd* does produce these compounds. While we do not know if *Axd* produces these compounds in nature to control its or other bacterial density, we did conduct a series of coculture experiments where *Axd* was grown in the presence of other bacteria, namely, *Staphylococcus aureus*, *Pseudomonas aerunginosa*, and *E. coli*. In all cases, *Axd* did not grow well in coculture with these bacteria and growth of the other bacterial species was also decreased.

While we continue to assess the impact, or risk of introducing *Axd* into natural ecosystems, we also must assess the same responses of *Axd* in terms of strain efficiency. Energetic costs associated with survival and nutrient acquisition may effect antibody gene expression, for example. Therefore, we first designed an assay to semi-quantitatively measure antibody gene expression using Reverse Transcription PCR and Real Time PCR. Studies are currently being conducted that measure antibody production by *Axd* when in different plant xylem. This information will assist us with optimizing our Symbiotic Control agent and provides a useful tool for risk assessment.

Regulatory Issues

The regulatory activities associated with development of transgenic pink bollworm for area-wide control strategies was described by Miller and Staten,[38] and Miller,[39] and are summarized in Table 1, taken from a PowerPoint presentation developed by Robert I. Rose, formerly a regulatory official with EPA and then USDA-APHIS before he retired December 2004. Dr. Rose is now a regulatory consultant specializing in biopesticides.

The regulatory activity associated with the Chagas disease symbiotic control project is largely anecdotal because there are no public outlets for documenting regulatory activity except for publication of environmental assessments in the Federal Register; but EAs summarize the biology of the regulatory object and do not necessarily describe the regulatory activities themselves. Thus researchers seeking permits and registrations are forced to reinvent the wheel each time a new case is posed unless they hire a consultant like Bob Rose.

When we first asked for permission from the Environmental Protection Agency to inject commercial grapevines with a genetically marked *Alcaligenes xylosoxidans* var. *denitrificans* (*Axd*) endophytic symbiont, they did not have a section that dealt specifically with symbionts, whether genetically modified or not. EPA did have a section that specifically dealt with the finished symbiont designed to secrete a reagent meant to control a pathogen, such as *Xylella fastidiosa*. EPA called our symbiont, *Axd*, a "microbial pesticide," even though the word pesticide is taken completely out of context in this application and perhaps microbial antibiotic is closer to the truth.

The EPA promptly responded to the first application for field trials, but required us to burn the grapevines as the conclusion of the trials.[39] We found this extremely odd since the Biosafety Committee at UC Riverside had already given us permission to use genetically modified *Axd* in out laboratory at BL-1 level. This level allows the bacteria to be used in High School Biology laboratories. Thus it is difficult to escape the impression that the requirement to burn the grapevines was overkill.

Results of the first year of field trials (2003) showed that genetically marked *Axd* (with a *Ds*Red gene inserted, therefore nicknamed "R*Axd*") did not survive in the xylem fluid of grapevines in commercial vineyards. We found,[40] in fact, that R*Axd* preferred to colonize the xylem of citrus far more than grapevines. Poor colonization of grapevines by *Axd* was independently confirmed by Steve Lindow (personal communication).

Since GWSS prefer citrus over grapevines, and because we normally collect GWSS on citrus, it made sense that the GWSS to isolate endophytes would reflect the normal complement of the xylem of citrus host trees. This would have to be taken into consideration for later application strategies, but for regulatory purposes, a poor colonization of grapevines from injections of R*Axd* would seem to introduce a further protection and safety layer. This was ignored by EPA who continued to insist on burning grapevines for a potential third year (not funded as it turned out) of field trials.

During the R*Axd* field trials from 2003-2005, the "Glofish"[41] was introduced. Glofish is a freshwater zebrafish, *Danio rerio*, originally found in the Ganges River in East India and Burma.

Table 1. Regulatory actions on transgenic pink bollworm

Action	Start	End	Time Lapsed	Result
Movement to Phx	1 Sep 1998	8 Mar 1999	6 months	Permit issued
Contained outdoors	29 Jan 2001	1 Oct 2001	8 months	Permit (and EA issued)
Movement to Phx	6 Dec 2002	22 Jan 2003	<2 months	Permit issued
Contained outdoors	14 Apr 2003	14 Jul 2003	3 months	Permit issued
Movement to Phx	1 Dec 2003	4 Feb 2004	2 months	Permit issued
Field release	5 Jan 2004 '	Cancelled	6 months	Withdrawn
Contained outdoors	27 Apr 2004	14 Jun 2004	1 2 months	Permit issued
Field release	8 Apr 2005	Pending	10 2 months	*Pending
Contained outdoors	25 Apr 2005	2 Aug 2005	3 2 months	Permit issued
Contained outdoors	28 Apr 2005	2 Aug 2005	3 months	Permit issued

*Environmental Assessment was posted (12 Feb 06) 8 months after it was written

Once genetically altered with a *Ds*Red gene, they appear red in normal room light and glow red under ultraviolet light. A Texas company asked for permission to sell the Glofish out of pet stores as an aquarium novelty. All of the federal regulatory agencies realized they did not have regulations that dealt directly with this case and waived review. California was the only state whose Fish and Game Commission denied permission to sell the Glofish. Their reason given was "I think selling genetically modified fish as pets is wrong."

California is not the only place to ban sale of the Glofish. There is no science in this conclusion by the California Fish and Game Commission, just a value judgment. While two of the three commissioners are entitled to their private opinion, they have made it pubic policy by this choice.

One might think that an aversion by the general public to the word "recombinant" or genetic "engineering" might be behind regulatory reticence. Transgenic crops are now an accepted part of agriculture, yet certain groups remain vocal in opposition. Mendocino, Marin and Trinity Counties in Northern California have voted to ban all transgenic crops (2005). Other Counties (Sonoma and Ventura) notably voted down a ban (also 2005). So there is clearly a difference of opinion in California amongst the voters.

It turns out that public influence of regulatory activities has more to do with scientific peer pressure than the public at large. Two pertinent studies[42,43] from the National Academy of Sciences appeared in 2004. The first was commissioned by the California Grape and Wine industry for the purpose of prioritizing funding.[42] On page 109 of that report the study group concluded that symbiotic control using recombinant had a limited chance of success due to the technical difficulty, operational difficulty and regulatory difficulty.

The head of the review committee, Jan Leach, captured the size of the threat to vineyards posed by the combination of GWSS and *Xylella* in the preface.[42] She admitted that her own experience suggested that "… breeding for resistance is the most economically feasible and environmentally sound approach to disease management." However, the report offered no clues to an eventual solution and at the same time the industry was warning researchers that transgenic grapevine solutions were not going to be tolerated. Indeed, if all of the research that was highly recommended in the report was fully funded and fully successful, in ten years California would still have Pierce's disease and the industry would still not know what to do about it. There seems to be a lack of appreciation for the enormity of the problem and lack of appreciation that traditional methods are inadequate.

Because existing technology is incapable to being used to stem the threat of Pierce's disease (or not allowed to bear, if the powerful tool of transgenic grapevines is off the table), one is forced to consider new technologies. Thus it seems obvious that the difficult work must be done to perfect new technology.

The second NAS study[43] largely concluded that nothing was known of the long-term consequences of release of genetically modified organisms other than plants. That seems fairly obvious since they have not been developed before. Caution in introducing new technology is always a wise course, however, laboratory studies never duplicate natural effects and no amount of laboratory data will anticipate all that might happen. At some point transgenic animals will have to be introduced simply because there are no other options.

Conclusions

Biotechnology offers new solutions to existing and future pest problems in agriculture including, for the first time, possible tools to use against insect transmitted pathogens causing plant diseases. Although the regulatory apparatus necessary to deal with the new strategies is in place, progress is slow partly because of the novelty of the application. Transgenic organisms developed through biotechnology are not the only examples facing difficulty; even the use of symbiotic bacteria that are selected by traditional nontransgenic methods is being delayed.

Conditional lethal pink bollworm strains are currently being held in quarantine at the USDA-APHIS laboratories in Phoenix, AZ. When these strains are mass-reared the lethal gene

expression is suppressed by a tetracycline repressor element that activated by the presence of chlorotetracycline, a normal component of the mass-rearing diet. Once removed from the tetracycline diet, the lethal genes are passed on to offspring when ordinary lab-reared pink bollworms mate with special lethal strains. Lethality is dominant (one copy sufficient for lethality), expressed in the egg stage and affects all eggs (100% lethal expression). The strategy first described as Autocidal Biological Control[16] over ten years ago. This technology is very close to completion for use in controlling pink bollworm. The initial investment by the California Cotton Pest Control Board is an outstanding example of research partnerships between agriculture industry, the USDA and land grant universities.

Symbiotic control was borne from the fertile imagination of Frank Richards at Yale Medical school, called paratransgenesis by David O'Brochta (University of Maryland) and has been translated from medicine into agriculture as the latest example of biotechnology innovations. The possible uses of this new technology will grow as it becomes applied to pest and disease complexes.

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