Perspective

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Delivery

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Abstract

Enthusiasm greeted the development of synthetic organic insecticides in the mid-twentieth century, only to see this give way to dismay and eventually scepticism and outright opposition by some. Regardless of how anyone feels about this issue, insecticides and other pesticides have become indispensable, which creates something of a dilemma. Possibly as a result of the shift in public attitude towards insecticides, genetic engineering of microbes was first met with scepticism and caution among scientists. Later, the development of genetically modified crop plants was met with an attitude that hardened into both acceptance and hard-core resistance. Transgenic insects, which came along at the dawn of the twenty-first century, encountered an entrenched opposition. Those of us responsible for studying the protection of crops have been affected more or less by these protagonist and antagonistic positions, and the experiences have often left one thoughtfully mystified as decisions are made by non-participants. Most of the issues boil down to concerns over delivery mechanisms.

Keywords: genetically modified organism; organic farming; pink bollworm eradication; sterile insect techniques; systemic insecticides

1 INTRODUCTION

There are many types of delivery. There is newspaper delivery by the paperboy, delivery of a baby by a mother and delivery of 'the goods', which is an expression that broadly means the ability to perform a job well. If someone does something well, you can say they have 'delivered the goods', which can mean anything from a concrete task well done to a successful speech drawing rave reviews. Christians regularly plea 'deliver us from evil', a request for heavenly transport to a safer place. In all these examples, delivery is a good thing.

Most of us can work in cities because a few of us choose to remain on farms and produce the food the rest of us eat. Arguably, you could say that we as an agriculture-based society have not been able to deliver the goods when it comes to pesticides. To me, one of the most interesting things about Rachel Carson's stance in the aftermath of her book *Silent Spring* was her criticism of modern chemical control as being bad and inefficient and her subsequent claim that we were 'capable of much greater sophistication in our solutions of this problem'.[1] It is too bad that Ms Carson is not alive today to witness the ravaging that some of these greater sophisticated solutions have received from the movement she started.

It has been estimated that somewhere less than 5% of an applied dose of insecticide reaches an insect pest when the insecticide is sprayed on an infested crop. Of course, the actual amounts depend very much on the crop, the pest insects and the prevailing conditions. This waste is the inefficiency Carson mentioned. Defenders of chemical pest control stress that considerably smaller amounts of modern insecticides are used for control compared with the first generation of synthetic insecticides. DDT sprays were measured in kilograms per hectare, but now we give amounts in grams per hectare of active ingredient. Moreover, modern insecticides are largely more selective than ever before, so they are safer to vertebrates, and non-target effects are less obvious.

Still, even given the obvious value of insecticides and other pesticides to modern agricultural crop protection, we can and should more actively seek ways to improve the delivery. Thus, this perspective article is not about insecticides; it is about the public attitude and delivery of insecticides and transgenic insects. The best early summary of delivery strategies of genetically modified insects was that of Braig and Yan.[2]

2 GENETICALLY ENGINEERED PLANTS AS A DELIVERY STRATEGY

Genetically engineered plants can produce insecticides or other control molecules [thinking of the Rothamsted genetically modified organism (GMO) wheat with aphid alarm pheromone] such as the delta endotoxin gene from the entomopathogenic soil bacterium *Bacillus thuringiensis*. This process is 100% efficient in delivery because most parts of the plant contain the toxin and the latter is not spread into the environment in any significant amount. Attempts were made a few years ago to point out that pollen carrying the toxin could affect beneficial or other non-target insects, but the amounts were small enough to be lost in the environmental 'noise' among other lethal factors.[3]

3 SYSTEMIC DELIVERY

Another form of delivery of insecticides that is more efficient than spraying is neonicotinoids in drip irrigation. When an insecticide has physical properties allowing it to express systemic activity, it can be delivered in drip irrigation and is taken up by the roots of crop plants (and any weeds nearby) and transported via the xylem

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vessels to the growing parts of the plants. Once there, it is present when an insect with chewing or sucking mouthparts eats leaves or stems or penetrates the xylem or phloem compartments of plants.

Temik® (aldicarb) from Union Carbide was one of the first systemic insecticides to be marketed and widely used. Although initially successful at boosting yield and apparently rejuvenating the plants themselves, there was eventually a fall from grace. In a famous incident in the mid-1980s in California, aldicarb was applied illegally to a watermelon crop, for which it was never approved. It was reported that 998 people were subsequently hospitalized with poisoning symptoms after eating the tainted watermelons. Prompt diagnosis and treatment with atropine reversed the effects.[4] In spite of obvious benefits in crop protection when used properly, the mammalian toxicity of aldicarb could not be ignored, and use gradually declined. Bayer CropScience announced plans to discontinue the manufacture of Temik (http://deltafarmpress.com/management/how-will-loss-temik-affect-insect-nematode-control).[5]

4 DELIVERY BY SEED COATING AND COLONY COLLAPSE DISORDER

Anyone with access to the media over the past 8 years has been informed about the ongoing crisis of mysterious European honey bee losses. It is mysterious because the cause is not known with certainty[6] and could indeed be due to many factors. Whenever an unexplained event such as colony collapse disorder (CCD) occurs, pesticides seem to be blamed almost by default until proven otherwise. In this case the broad class of neonicotinoid insecticides are blamed, with studies claimed as evidence for effects on bees,[7–9] others showing evidence against[10] and still others calling for more study.[11–13]

One side lobbies to ban the insecticides until conclusive proof is produced that they are safe, while agrochemical industry advocates warn of crop losses if their use is curtailed. Unmentioned in the debate is the quiet fact that the neonicotinoid category of insecticide has displaced carbamate, organophosphorus and pyrethroid insecticides combined to become the largest category of insecticide in total global sales (Sparks T, Dow AgroSciences, unpublished, 2012). Thus, banning these insecticides would have consequences.

Recently, an industry–grower–government consortium was organized to solve a known delivery problem with neonicotinoids.[14] Note that neonics were recently 'banned' in the EU because of the perceived effect on bees. While small amounts of systemic insecticide delivered in drip irrigation are found in nectar and pollen of a plant, it has become common practice to coat seeds with the neonicotinoids at planting. Moreover, because machine planting of seed is dry, the insecticide is mobilized as a dust during the process. The amounts have been measured, and drift is known to occur. Thus, this is a delivery problem, and some consortium members expressed optimism that a new formulation could largely eliminate dust drift. This is an excellent example of collaboration (as shown in the quote below[14]), which should be encouraged:

> Stakeholders seek to reduce the amount of pesticide-contaminated dust generated during corn planting. Corn Dust Research Consortium, collaboration formed by pesticide industry, seed companies, farm equipment manufacturers, corn growers, beekeepers, conservation groups, and researchers for academia and the US and Canadian governments.

The neonicotinoid insecticide imidacloprid has been protecting grapevines in Temcula, California, with a one-time treatment in early season since an epidemic occurred at the end of the 1990s. This is currently the only known treatment to prevent Pierce's disease (PD) caused by the bacterial pathogen *Xylella fastidiosa* transmitted mainly by the invasive leafhopper *Homalodisca vitripennis*.[15–17] Once a systemic insecticide is delivered via drip irrigation (a process known as chemigation), it remains in treated plants for several weeks. Area-wide control includes systemic treatment of citrus, a highly favored overwintering host for the vector insect.

When applied by drip irrigation, imidacloprid has been shown to affect natural enemies visiting the same plants.[18] Thus, the persistence and disposition in the plant, while preventing feeding by insects, can have effects on non-target organisms (I have often wondered whether this is due to reverse gutation – drops of water early in the morning in dry conditions – thus exposing the surface of the leaf to any systemic). We know that the insecticide is responsible for preventing Pierce's disease, based on the prevalence of the disease in some organic vineyards in the Temecula area, which are not allowed to use synthetic insecticides; this is worth checking, as many organic schedules use so-called 'natural insecticides', including, amazingly, spinosad (the myth that if it is natural it must be safe – like bubonic plague and aflatoxin – both really natural, but . . .). None of these seem to get the same degree of attention as synthetic materials, e.g. rotenone was still available for organic use until recently, even though it had been removed from normal insecticide use many years previously.

5 TRANSGENIC INSECTS AS A MORE SOPHISTICATED CONTROL METHOD

The so-called sterile insect technique (SIT) was developed some 50 years ago and was originally based on radiation to induce sterility.[19] In essence, the pest insect is converted into its own biological control agent. This is arguably the most sophisticated form of insect population control, but it is so expensive in operation that only the most compelling pests are realistic candidates. Mass rearing and daily releases of large numbers in target areas account for the cost, mostly in labor.

In practice, radiation has many side effects, with loss of fitness and performance, which are counteracted by inundation with daily releases of large numbers of SIT insects. The SIT strategy works best when the target insect is confined to a specific crop or area, can be mass reared and can withstand the radiation dose to compete for mating. So far it has been applied to Diptera and Lepidoptera, but it was found to be unsuitable for boll weevil because the radiation levels necessary for sterilization were too debilitating to that insect.[20] The reasons for this could include destruction of symbiotic bacteria in the weevils by radiation, from which they cannot recover, but this has not been investigated yet.

The California Cotton Pest Control Board decided in 1988 to fund an effort to transform the pink bollworm *Pectinophora gossypiella* genetically. This was after the genetic transformation of *Drosophila melanogaster* had been reported by Rubin and Spradling[21] using a transposable element named P. The cotton industry leadership wanted a strain of pink bollworm with a conditional lethal gene that could allow mass rearing but be capable of outcrossing and back-crossing to improve fitness and reduce the numbers required for daily release, thus greatly reducing costs.

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It took until 1998, fully 10 years, from the initial request to the final transformation of pink bollworm.[22] In the meantime, a second genetic transformation was reported for Mediterranean fruit fly.[23,24] This time, another transposable element was used, named *Minos*. Even before the genetic transformation of pink bollworm was achieved, the regulatory process appeared to be unprepared. This was documented in a meeting involving the Pew Foundation, and their report was published in 2004.[25] This inadequacy was certainly our experience,[26–33] expressed in the form of delay needed to determine the correct course of action by regulatory agencies facing requests for permits for field trials of GMO pink bollworm and threats of law suits if they were issued.

The regulatory agency responsible, USDA-APHIS-BRS (Biotechnology Regulatory Services), eventually did issue permits for field trials of transgenic pink bollworm.[34,35] The Center for Food Safety in Washington, DC, withdrew the threat of a law suit once they were satisfied that BRS had completed all legal requirements for oversight with the publication of an Environmental Impact Statement.[36]

6 THE ORGANIC GROWER INDUSTRY IS AGAINST SUSTAINABLE METHODS

In the final analysis there was insufficient motivation to push for final use of the transgenic pink bollworm with a lethal gene inserted.[37] The US cotton industry could be viewed as a cautious protagonist. Although the new biotechnology was in place at a significant cost, when it was proposed to use just the genetically marked strain of pink bollworm as a means of distinguishing SIT released insects from wild types caught in monitoring traps, the staff of the National Cotton Council contacted the National Organic Program and asked about using GMO pink bollworm in the eradication program.

Shannon Nally, Agricultural Marketing Specialist, USDA National Organic Program, replied:

> According to the National Organic Program (NOP) regulations, an organic product must be produced and handled without the use of excluded methods. (7 CFR 205.105(e)). Excluded methods are defined by the NOP regulations to include, 'A variety of methods used to genetically modify organisms ... by means that are not possible under natural conditions or processes. ... Such methods include ... recombinant DNA technology (including gene deletion, gene doubling, introducing a foreign gene, and changing the positions of genes when achieved by recombinant DNA technology). ... (7 CFR 205.2).

The intent of the NOP regulations is to prohibit the use of excluded methods in all aspects of organic production and handling, including pest control. Therefore, the release of the transgenic sterilized pink bollworm moths over organic cotton acreage as part of a pest control regimen would adversely impact the farm's organic certification. In addition, an organic cotton operation would need to maintain an adequate buffer area if transgenic sterilized pink bollworm moths were released over adjacent land. If sterilized pink bollworm moths are to be released within a region, the release of non-transgenic radiation sterilized insects over the organic cotton acreage would be an acceptable alternative.

National Cotton Council Staff members were told that, if one pink bollworm were to land on an organic cotton crop, that crop would lose certification because the National Organic Standards explicitly exclude the use of any GMO. The organic grower would not have to go through the 3 year recertification procedure if one crop were condemned by such contamination, but the crop that had come into contact with GMO pink bollworm could itself not be sold as organic. The default position of doing nothing was embraced rather than risking possible bad publicity and liability over the issue or jeopardizing federal support for other programs (Parker D, private communication, National Cotton Council, 3 May 2013).

Thus, the organic grower industry has placed itself in direct opposition to using the most sustainable and environmentally friendly insect control method ever invented (the use of GMO insects in the SIT strategy). Yet this is exactly what they claim to support. It is a dilemma created by deciding arbitrarily what is organic. The organic industry benefits when pink bollworm is eliminated from the entire US and adjacent Mexican cotton-growing areas. It is similar to the 'logic' that boxed the California Fish and Game Commission into a corner in denying sale of 'Glofish'.

Glofish is a transgenic tropical zebra fish sold as a novelty in 49 US states. Sale in California was denied with the statement, 'we shouldn't be doing that'. When someone realized how arbitrary that explanation was, attorneys promised permission for such sales in California if the industry would conduct an environmental impact study. Such a study could cost millions of dollars, in spite of all federal regulatory agencies finding nothing to regulate.

The zebra fish is not a pathogen (CDC regulated), not a food or crop (USDA regulated) and not a drug (FDA regulated), and nor can it survive outdoors in any US state if released (EPA or Fish and Wildlife regulated). Thus, it is a completely non-regulated item. So asking for an environmental impact statement for something that has no known environmental impact is rather brazen, given that there is no federal agency to receive or evaluate such a report. Nevertheless, the Glofish industry and anti-GMO advocates have come to a tacit understanding.

7 PINK BOLLWORM ERADICATION PROGRAM

At great expense and trouble, the US cotton industry, growers and governments have mounted a program to eliminate the pink bollworm from the Unites States and northern Mexico. This effort includes the states of Texas, New Mexico, Arizona and California and the adjacent cotton-growing areas of Mexicali, Sonoyta and San Luis in the west and Chihuahua in the east, adjacent to southern New Mexico and southwest Texas.

The last pink bollworm adult was caught in a pheromone trap in the program area during May 2012, as shown in Table 1. Thus, most of the cotton-growing season of 2012 was pink bollworm free. The last moths to be captured were in the Yuma/San Luis area astride the Colorado River near the Arizona and Mexico border. This area has been the target of sterile insect releases. The accepted time period for declaring an insect removed from a certain target area is 3 years without catching an adult in a pheromone trap. This is based on old literature references that claimed the pink bollworm could diapause for at least up to two and possibly three years.

Talks are already planned by industry representatives, private citizens and government personnel, involving the fate of the pink bollworm rearing facilities that are located in Phoenix, Arizona. If

Table 1. Pheromone trap catches of adult male pink bollworm *P. gossypiella* for the last 2 years of the pink bollworm eradication program[a]

Location	2001	2002
Arizona Central	566	0
Arizona Northwest	17	0
Arizona Yuma	0	1[b]
California Bakersfield	1	0
California Imperial Valley	1	0
Chihuahua, Mexico	9	0
Mexicali, Mexico	401	18[b]
New Mexico	0	0
San Luis R.C., Mexico	315	7[b]
Sonoyta, Mexico	0	0
Texas	60	0

[a] Source: Staten.[38]
[b] Last adults capture in May 2012.

the pink bollworm is eliminated from the targeted areas, the cost for operating the rearing facility could no longer be justified.

8 ERADICATION OF BOLL WEEVIL

Another cotton pest is currently the target of an industry–government partnership for elimination from US cotton fields – the infamous boll weevil, *Anthonomus grandus grandus*. Already cleared from most of the invasion range from Texas to the east coast, the pest stubbornly resists final elimination. Reasons for this include wild cotton hosts away from commercial cotton crops, which provide a continuing harborage. Ironically, the boll weevil was never a viable candidate for SIT because the radiation levels necessary for sterilization are too lethal.

However, if this insect were the target of a GMO SIT effort, it is possible that SIT could be mounted and final elimination could be achieved. Sadly, however, such a plan would again run foul of the National Organic Program, unless that program worked out some waiver mechanism compatible with the national interests.

9 DENGUE CONTROL WITH TRANSGENIC MOSQUITOES

In the fall of 2010, the British company Oxitec, Ltd (Oxford UK), reported on a field trial conducted in the Cayman Islands with dengue mosquitoes, *Aedes aegypti*, that were genetically modified to carry a lethal gene. Male mosquitoes, which do not take blood meals, were released into the environment and, according to the Oxitec summary, caused an 80% reduction in the native population as compared with non-release areas. It was suggested that the 20% that was recovered in the release area were mainly fly-ins from non-treated areas. Readers can readily find much reported information online about these activities. Just two URLs will be provided here, one from an antagonist and the other from the protagonist: Genewatch, December 2010, http://www.genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/Oxitecbrief_fin.pdf; Oxitec press release, September 2012, http://www.oxitec.com/press-release-oxitec-and-mrcu-report-80-suppression-of-a-dengue-mosquito-population-in-grand-cayman-by-release-of-engineered-sterile-male-mosquitoes-nature-biotechnology/.

Also, the 29 March 2013 issue of *Science* contains a commentary on the subject, 'Genetically modified organism policy', in a feature called *The Buzz*. Divergent viewpoints from previous issues are presented.

When male *Aedes aegypti* mosquitoes are released into the environment in this manner, they can certainly obtain moisture and survive for a certain period of time, but they do not take blood meals. They also seek females of the same species for mating and pass on the lethal gene designed by Oxitec. This release strategy is similar but not identical to SIT based on radiation to induce lethal genes. Thus, the insect is converted into its own biological control agent, but must be released daily to help ensure disruption of mating between wild-type partners.

Oxitec has conducted trials in partnership with the Cayman Islands, Malaysia and now Brazil. For an extensive recent analysis of the operations in Brazil, see Reis-Castro and Hendrickx.[39] Their article reports that dengue costs the Brazilian government about €400 million yearly. It is described as a 'very serious public health issue'. It is also described as severe because of the geographical spread and a recent increase in dengue hemorrhagic fever (DHF), the most dangerous manifestation of the virus infection. As there are no known vaccines for the four dengue virus serotypes, the only other strategy is spraying insecticides to control larvae that breed in pools of water. The latter represents a classic delivery problem rife with inefficiency because, unlike the SIT strategy, insecticides do not seek out the mosquitoes.

The Oxitec approach, which they call RIDL (Release of Insects with Dominant Lethals), is equivalent to dropping insects destined to die into a designated area. Detractors point out data from Oxitec showing that a very small percentage of the RIDL mosquitoes can survive the larval and pupal stages and emerge as adults. Oxitec counters that the number of survivors is insignificant.

The example given above of dengue control with conditional lethal genes is an example of the most sustainable and ecologically friendly delivery method ever yet invented.[40,41] It is equivalent to dumping dead insects onto the sidewalk. There are no side effects, no non-target effects and no resistance that we can conceive of yet, although it was pointed out by Jorge Hendrichs of the International Atomic Energy Agency recently that the single conditional lethal gene is more prone to selection for resistance than radiation-based SIT because radiation induces multiple lethal gene effects that are much more difficult to overcome in resistance selection. Thus, GMO insects used in the SIT strategy are candidates for what Rachel Carson sought – a more sophisticated solution to the pest insect problem.

The opposition to the use of GMOs in the SIT strategy makes several points. The main one involves transparency, with complaints about lack of information. Critics demand to see raw data to judge for themselves about the efficacy of the strategy. There are also calls for more study about long-term effects. While the call for more study seems neutral and reasonable, there is seldom a suggestion for what kind of study is needed; indeed, the danger of using GMOs in SIT is never defined or addressed. What exactly are you supposed to measure when there are no toxic effects, no non-target effects and no residue to find? If the approach had at least some toxicity or other measurable property, it would be much easier to define in terms of limits. This approach has none of those.

Critics are not focused on SIT but on GMOs. Some are bothered that mankind inserting genes into organisms for whatever purpose is 'unnatural'. This view puts mankind apart from nature.[30] There are endless arguments about where the genes come from, and that such a thing could never have happened in nature. There seems to be a lack of awareness about the latest advances in symbiosis and microbiology.

10 HUMAN BEINGS ARE STEWPOTS OF MICROORGANISMS

From symbiosis we learn that all higher organisms are 'stewpots of microorganisms'.[42] As the polymerase chain reaction discovery has enabled potential identification of all microbes (viruses, bacteria and fungi), symbiosis has revealed a rich participation in the biology of organisms by microbes that has hitherto been ignored. From the earliest studies of DNA manipulation, bacteria were known to be 'competent', meaning raw DNA can be taken up from the environment. Once inside the bacteria, the DNA can be incorporated into plasmids or chromosomes.

Thus, the claim that genetic engineering to move genes from abroad into a given organism is novel or has not been done before is naive because bacteria rely on this mechanism to acquire new traits to cope with stress or changing conditions. Thus, the emphasis on the process of moving DNA detracts from the more crucial mechanism, which is selection. It does not matter so much how DNA moves around, it matters what combination of DNA is produced and how this is moulded by selection. Selection implies new combinations in sexual reproduction, new mutations and new symbiotic relationships.

11 GENETIC ALTERATION AS A ROUTINE FUNCTION IN GENOMES

Genetic modifications occur on a regular basis. My current favorite example is that transposable elements play a key role in brain function in insects.[43] The transposons are deliberately mobilized for insertion into new places in the chromosomes of specialized cells of the brain region of *Drosophila melanogaster* known as the mushroom body which is responsible for memory and plays some role in memory enhancement. We find increasingly that DNA modification happens constantly as a response to stress or need for adaptation.

This article[43] also reminds us that 45% of the human genome was found to comprise transposable elements. These are mobile pieces of usually short DNA (a few thousand base pairs long) that move around or mutate to inactive forms and can become what has been called 'junk' DNA that accumulates in chromosomes. The amount of transposable elements in *Drosophila* is 15–20% of the genome. Thus, a good guess is that these transposable elements play some role as yet unknown beyond the memory trick just reported. My understanding is that these play a crucial role in gene up- and downregulation.

As mobile elements have been found on viruses, we suspect that they can travel between unrelated species via viral infections. This further emphasizes the ability of DNA to move between chromosomes in distantly related species. Thus, DNA moves, and the argument that the manipulations by scientists in laboratories are something that could never happen in nature is untrue? The real emphasis should be on selection not movement of DNA.

The one message of the organic farming movement that does resonate is the concern over human alteration of the environment. Here we should all be on the same page and be proactive, as warned by Amato *et al.*[44] and Zachariah *et al.*[45]

12 CONCLUSION

The organic farming movement has an honest desire to return to a place that is presumed to be better than the present world. Arbitrarily defining some current farming methods as unnatural is to presume that anything mankind does is somehow unnatural and that mankind is somehow apart from nature. The organic movement also claims to embrace the most sustainable and ecologically friendly approaches to agriculture. And yet they reject the one scientific advance (the use of GMO insects in the SIT strategy) that is the most sustainable and ecologically friendly method yet invented. It leaves no footprints. Use of GMO insects in SIT is a powerful technology, one that is not duplicated by any other method. To deny its use for arbitrary reasons is to shoot oneself in the foot and, what is worse, shoot everyone else in the foot as well.

This issue needs to be part of a national debate to create waivers for those conditions and situations where it is in the best interest of everyone to focus on a major pest that has no respect for any part of agriculture, organic and non-organic alike.

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