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Insecticide Resistance after *Silent Spring*

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Combating insecticide resistance is a continual challenge for the preservation of both traditional and transgenic crops.

Rachel Carson's book *Silent Spring*, published 50 years ago (1), eloquently awoke the public to the manifold dangers for the environment and human health posed by the wanton use of chemical pesticides (2). Carson argued that in addition to their many harmful ecological effects, chemical insecticides ultimately undermine sustainable pest management: They kill the parasites and predators that formerly held many pests in check, while the pests themselves become resistant and require ever-higher amounts of sprays for their control. Since the publication of *Silent Spring*, more than 450 arthropod species have been reported with resistance to one or more pesticides (3). Yet over the same period, a paradigm shift in dealing with this global problem has also occurred.

Resistance, defined as the heritable decrease in a population's susceptibility to a toxin to which it is exposed over successive generations, is an example of evolution by natural selection. The intensity of selection can be controlled by varying the insecticide and reducing the frequency and intensity of application. A variety of different chemical classes has been developed, targeting a range of biological targets in the insect. Yet because the number of targets is still limited and new targets are not resistance-proof, prolonging the useful life of existing insecticides by judicious use has increasing priority in their commercialization (4). Because pesticide use is difficult to control on a global level, resistance management programs must be locally suitable, economically feasible, and voluntarily adopted by growers.

Pesticide resistance often results from gene regulatory changes, which lead to an increase in the efficiency of one or more physiological systems used by the insect for detoxification: oxidation, conjugation to hydrophilic compounds, and excretion. Constitutive up-regulation of these detoxifying enzymes is most common, although gene amplification is another mechanism for increasing the amount of protein available to inactivate the insecticide. In such cases, general inhibitors of classes of detoxifying enzymes may be used to counter the increased detoxification ability.

Rarely, a single mutation can confer a novel detoxifying ability, such as the substitution of an aspartate for a glycine in a carboxylesterase of the Australian sheep blowfly, converting it to an organophosphorus hydrolase (5). More commonly, single mutations reduce the sensitivity of the insecticide's biochemical targets, including enzymes and ion channels in the nervous system. In these proteins, which are essential for life, only a very few mutations are compatible with biological function; these mutations are often found in many

different species exposed to the same insecticide, representing multiple cases of parallel evolution. Thus, engineering modified insecticides that are more potent on the altered target sites could be of general benefit. This predictability at the level of target-site mutations is, however, often frustrated by the presence of additional, more diverse detoxifying mechanisms in the same species.

A good example is the complexity of resistance in mosquitoes, which are vectors of malaria and other diseases. Gene amplification causing overproduction of organophosphorus-inactivating carboxylesterases has spread worldwide in *Culex* species (6). Some *Culex* species have evolved further to replace this energetically costly overproduction of protein by mutation and gene duplication of the target, acetylcholinesterase (AChE) (7). Bariami *et al.* recently used microarrays to show that increased expression of a CYP9J-type P450 and an ABC transporter, partly due to gene amplification, may underlie resistance to pyrethroids in populations of the dengue vector *Aedes aegypti* (8).

The aphid *Myzus persicae* is another serial offender. It first evolved organophosphorus resistance by making up to 80 copies in its genome of a pair of esterase genes, resulting in esterase protein up to 1% of body weight (9). This expensive overproduction is switched off by an unknown mechanism when the aphid demethylates these gene copies. Target-site mutations in AChE compensate for this loss; sodium channel mutations additionally confer pyrethroid resistance. The aphid counters the newer neonicotinoid insecticides by multiple duplications of the CYP6CY3 P450 gene (10) and a target-site mutation in a subunit of the acetylcholine receptor (11); this combination of mechanisms is especially potent.

The spider mite *Tetranchyus urticae* is notorious for rapidly evolving resistance, spurring the development of novel chemicals for control. Resistance to binfenazate has been shown to result from four mutations in the mitochondrial DNA that encodes cytochrome b; these mutations occurred at positions that are otherwise completely conserved across eukaryotes, identifying the target site of this new pesticide (12). This unusual, maternally inherited resistance responds extremely rapidly to selection in the field. Recently, Van Leeuwen *et al.* used the mite's genome sequence to investigate resistance to etoxazole, which inhibits synthesis of the chitinous exoskeleton of arthropods. By selecting a mixed population and tracking frequency changes of more than 700,000 polymorphisms, the authors identified a genome region that contains a mutated chitin synthase gene, with a single amino acid substitution conferring resistance (13).

Toxicity to non-target organisms and reduced effectiveness on resistant pests constituted two separate threads of the argument against pesticides in *Silent Spring* (1). Remarkably, these ideas intersect on the genetic model *Drosophila melanogaster*, which, despite not being a pest, has received enough incidental environmental exposure over the years to develop resistance to several older insecticides. Dieldrin-resistant flies from the wild enabled the first identification of the *Rdl* mutation in the GABA-gated chloride channel (14), subsequently found in many pest species. One cause of DDT resistance is the insertion of a

transposable element in the promoter of CYP6G1 (15), leading to overexpression of this P450 enzyme, which detoxifies DDT. *Drosophila* later attained even higher DDT resistance by two additional insertions and a gene duplication, revealing an ongoing process of multiple adaptive steps (16). To identify the targets of newer insecticides, Perry *et al.* have used mutagenesis of *Drosophila* to create strains resistant to spinosad or neonicotinoids; they pinpointed the acetylcholine receptor subunits that are most sensitive to these toxins (17).

The goal of reducing the use of chemical insecticides has spurred the search for biologically based alternatives, a strategy encouraged by Carson [chapter 17 in (1)]. Insecticidal protein toxins from the bacterium *Bacillus thuringiensis* (Bt) are now expressed in more than 58 million hectares of transgenic cotton and maize worldwide to deter lepidopteran pests (18). When Bt cotton was first introduced in the United States and Australia, government-mandated, industry-implemented resistance management plans were in place. These "high dose/refuge" strategies aimed to slow the process of natural selection, first by ensuring that transgenic plants expressed enough toxin to kill all but the most resistant insects, and second by providing non-Bt crops as "susceptibility refuges" on which Bt-susceptible pests could develop to adulthood and mate with the relatively few survivors from the Bt crop. These strategies to delay resistance are working so far in most cases (19).

What if they fail? Estimation of the frequency of rare Bt resistance alleles before they become common enough to cause unsustainable crop damage can provide advance warning of developing resistance. Using methods based on the inbreeding of large field samples, Downes and Mahon have detected alleles for resistance to the Cry2Ab toxin at frequencies of 0.5% to 0.9% in two species of bollworms in Australia (20). When the resistance gene is known, DNA sequencing can also be used; Zhang *et al.* have correlated mutations in a 12-cadherin-domain protein with bollworm resistance to the Cry1Ac toxin in China (21). Modified Bt toxins have been engineered to circumvent this type of resistance and show promise on other Bt resistance mechanisms as well (22).

Co-expression of an additional toxin, Vip3A, with a different mode of action has been commercialized to delay pest resistance to transgenic crops; however, the Vip3A resistant allele frequency is already 2.7% in one pest, which is very high given that there has been no prior exposure to this toxin (23).

Forewarned by the long history of insecticide resistance, the deployment of transgenic crops for insect control has incorporated resistance management plans from the beginning. Unfortunately, this has not been the case for transgenic crops engineered for herbicide tolerance. Greatly increased spraying to control weeds in these new crops has led to a rapid rise of herbicide resistance in several weed species (24), and agronomists must now follow entomologists in learning the hard lessons of the past 50 years.

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Figure 1. Some mechanisms of insecticide resistance. Insects have evolved mechanisms to detoxify, reduce their sensitivity to, or excrete insecticides. Increased detoxification can occur by (A) gene duplication of carboxylesterase, which cleaves the insecticide, or (B) transposon insertion, causing increased transcription of P450, which hydroxylates the insecticide. (C) Point mutations in the target can reduce insecticide binding. (D) Increased transporter activity leads to faster excretion from the cell.