Chapter 18

Prevention Versus Remediation in Resistance Management

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"After the fact" remedial strategies are often ineffective, especially where resistance is widespread and/or refuges are large. Good pesticides are too often lost. The "it won't happen here" view accounts for the rarity of area-wide management strategies. The successful national example of abolishing agricultural use of DDT in Sri Lanka in favor of its use only in mosquito control precluded resistance until now. Preventive strategies must be immediately cost-effective, as well as useful in delaying resistance, or they will not be implemented. The tendency to cut dose rates is increasing resistances due to multiple-cumulative events (polygenic, amplifications, or sequential mutations within a gene). We have modeled alternating low with intermediate dose rates to delay both major gene and multiple cumulative-resistances as part of IPM. Such novel strategies must be verified with economic and pest control data to convince farmers that they can work.

Burgeoning Resistance - An Ever Increasing Problem

Resistances to pesticides are becoming more widespread, and more resistances are to be expected as more farmers use pesticides. The appearance of pesticide-resistant populations is not a developmental process and has nothing to do with developmental biology, as some specialists imply when they erroneously discuss "development of resistance." Resistant populations do not mysteriously "develop" but evolve according to evolutionary processes. We can try to modulate the rate of evolution, preempting its appearance, or wait and try to selectively rid our crops of resistant pest populations

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by remedial procedures after resistant populations evolve. This increases the need for both remedial strategies to overcome resistant populations that have already evolved, as well as more cost-effective preemptive strategies to prevent resistance from evolving. This is also termed "managing susceptibility".

The most pernicious cases will be of new resistances to herbicides and not to other pesticide groups for a variety of reasons:

(a) Herbicide usage (whether measured in expenditures, area covered, or weight of active ingredient) is increasing relative to other pesticides.

(b) Herbicide usage is increasing in lower value crops such as wheat and rice, especially in the developing countries. Resistances are following suit (*1-3*). There is a stronger likelihood to repeatedly use single compounds in these typically monoculture crops. Monoculture with one pesticide is a harbinger of resistance.

(c) Earlier single-target herbicides focused on a chloroplast genome coded target (*4*). Mutations to resistance were functionally recessive at field light intensities, as when there are susceptible plastids, they photogenerate large amounts of toxic oxygen radicals that cannot be detoxified. Individuals carrying large populations of resistant plastids are at a very low frequency. This resistance is inherited on a highly conserved gene, and most resistant individuals are rather unfit.

(d) In contrast to (c) there are many current, fashionable, low dose-rate, single-target herbicides where the resistance trait is rather fit, dominantly inherited, and at a high frequency (*5,6*). Thus, resistance is rapidly appearing to these herbicides, due to their large market share.

(e) Resistant populations are beginning to appear to herbicides that have been considered immune to such problems; the phenoxys and chloroacetamides, and other compounds in continuous heavy use for >25 years.

More cases of resistance are also expected to insecticides, as the number of targets of presently available compounds is small, and cross resistances are thus rampant. Still, insecticides are used in higher value crops where farmers have a greater stake in success and can afford a wider variety of pesticides in resistance management schemes. The fewest problems are expected with fungicides, as crop genetics and breeding are more often successful in overcoming pathogens than weeds or arthropods. Pathogens evolve resistance despite sophisticated breeding strategies, so breeders have the same problems as those engaged in rational pesticide use.

We can always expect new problems with pests that were never well controlled filling niches left vacant when successful pesticides eliminate the primary pests. We also have the pests that seem to have the ability to rapidly evolve resistance to every new pesticide developed for their control; e.g. the *Coccidiosis* pathogen of poultry (*7*), the Colorado potato beetle (*8*) and ryegrass species in wheat (*3*), and more recently *Echinochloa* spp in rice (*9*).

Reduced Pesticide Usage - Adding to Resistance Problems. There are increasing economic, regulatory, and consumer-induced pressures to decrease pesticide usage. This can be done in three ways (sometimes with more than one together), each with implications towards the evolution of resistance and its management:

Increased Abstinence. Growers can chose to apply pesticides less often, which can have a variety of effects. If the pesticide was not needed in the first place, as is too often the case, then the effect can clearly be positive as the farmer has met the economic and regulatory goals. The few resistant individual weeds may be successfully suppressed by the crop. All resistant pests will be subject to competition by more fecund susceptibles, if, and only if there is a substantial fitness differential between resistant and susceptible individuals. Abstinence also allows insect populations to be decimated by natural enemies that are suppressed when insecticides are used.

Abstinence can lead to problems when pest populations arise well above their normal levels. Evolution is a numbers game; if other parameters are equal, the more individuals present when a selector such as a pesticide is used, the more likely there will be resistant individuals present per unit area when pesticide treatments begin,

assuming a constant mutation frequency. The more resistant individuals present from the start the more rapid the evolution of predominantly-resistant populations. The initial frequency of resistance is a less compelling parameter in pests with high dispersion rates such as insects. When abstinence is to be used, it should be with good scouting, so that pesticides are used when the pests are above thresholds, and abstinence instituted when below.

Using New Low Rate Pesticides. The use of highly potent, low rate pesticides strictly meets the letter of regulatory fiats. Many new pesticides with long-lived residues do not meet the raison d'être for these regulations. Most low dose-rate pesticides have a single target, and it is easier to evolve resistance when only a single target need be mutates, than when many different targets need be mutated. Long residual activity generally increases selection pressure, enhancing the rate of evolution of resistance. Low pesticide rates clearly do not mean low selection intensity for resistance. Conversely, there is a tendency for authorities to demand the deregistration of older, higher dose-rate, often multiple-target pesticides, reducing the flexibility of farmers to mix or rotate them with low dose rate pesticides in well wrought IPM packages. Regulators must be made aware that such deregistrations can be counterproductive; resistance problems will become more rampant, and we will then have to return to the older pesticides to successfully produce crops.

Cutting Pesticide Rates Leads to Further Problems. Recently there have been increasing numbers of cases of resistance evolving where lowered rates of pesticides were used. When high dose rates are used, resistant populations often appear suddenly to the farmer (Fig. 1A), although actually there was a smooth exponential build up of resistant individuals beginning from some low frequency (near the mutation frequency) to resistant populations. When resistant populations appear seemingly without warning, all resistant members of the population are resistant to high dose rates, as seen in Fig. 1B. When a constant lower dose is used, one can select for any one of a plethora of polygenes. As there are many available, the trait is at a much higher frequency than single major genes for resistance. [Polygenes are used loosely herein to cover all multi-cumulative events including accumulating polygenes, as well as multiple mutations within a gene that incrementally confer increasingly greater resistance, as well as gene amplification. A better, but far longer and convoluted descriptor would be "incrementally increasing resistance caused by selection of cumulative, multiple, sequential, mutational events"].

Field data demonstrating such creeping resistance are shown in Fig. 1C. Each dot in Fig. 1C describes the average of a *Lolium* population found in an Australian wheat field. An analysis of such data (Fig. 1D) shows how the average level of resistance in populations is incrementally increasing throughout the population as a function of repeated low dose applications. In North America, where a 3 fold higher dose of the same pesticide was used, target site resistance evolved in three major pest species (*6,12,13*), including *Lolium*, (*6*) the same genus evolving polygenic resistance in Australia.

The nature of polygenic inheritance is such that there are small increments of increase in resistance in such a population (Fig. 1C,D). Perhaps the appearance of a measurable proportion of individuals with the first increment of resistance is delayed (as in Fig. 1C) until the first polygene for resistance has been sufficiently enriched in the population. Formally, at this stage we have a "single gene" resistance, albeit to a very low level of pesticide. After the first increment of resistance appears, some individuals can withstand the evolutionary pressure of higher pesticide doses, enriching more gene doses. Initial models on evolution of quantitative resistance to pesticides were described, but not fully developed (*14*), and "the impact of quantitative trait locus studies on evolution has yet to be felt" (*15*). Presumably this means that while there is considerable circumstantial and epidemiological evidence for polygenic controls, the genetic proofs are rare. There is evidence for polygenic inherited incremental increases in resistance to some fungicides (cf.*16-19*), insecticides (by gene amplification) (cf.*20-21*), herbicides (*22*), and gene amplification resistance to anti-cancer drugs (cf. *23*).

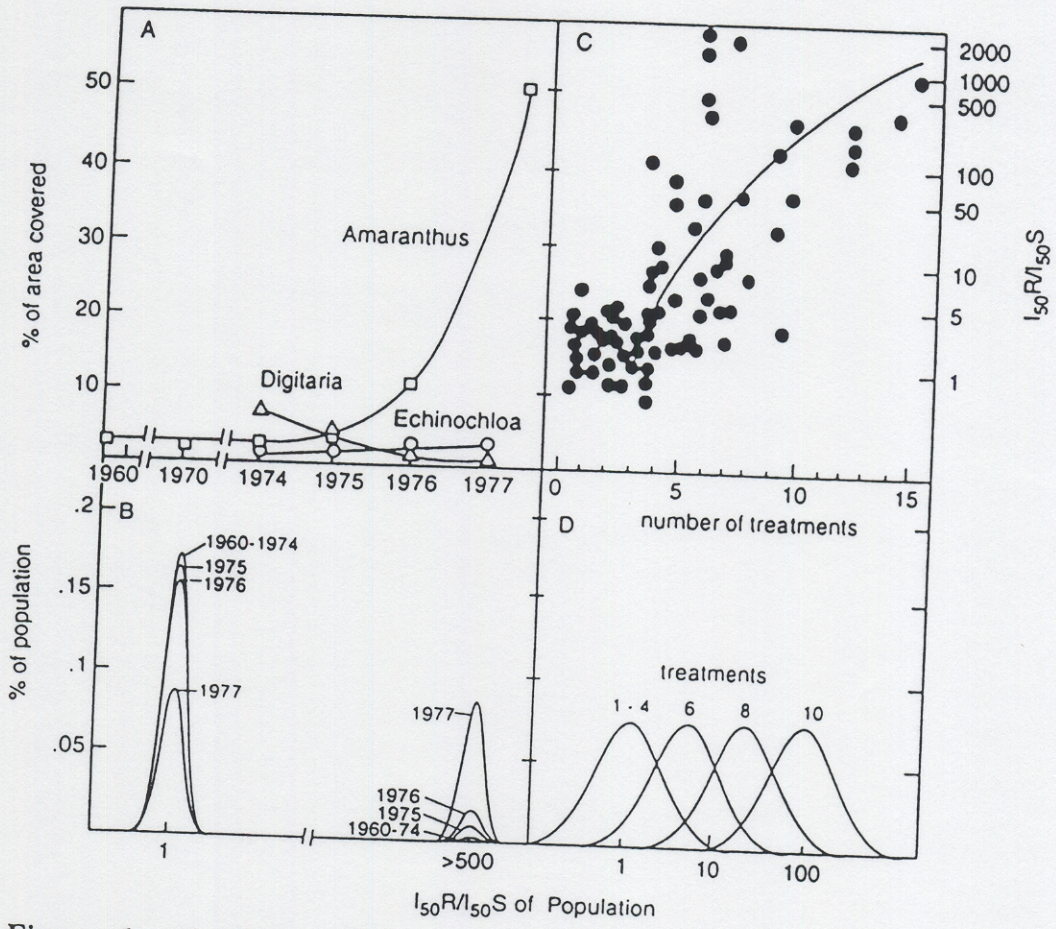

Figure. 1. "Sudden" appearance of major monogene resistance vs. slow incremental creep of polygenic resistance.

A. Actual field data on resistance showing changes in weed populations in a monoculture maize treated annually with atrazine. *Amaranthus retroflexus*, *Echinochloa crus-galli*, and *Digitaria sanguinalis*, the foremost weeds, were counted. The maize field was treated with atrazine from 1970 onwards. (Data are plotted from Table I in ref.*(10)*.

B. A population distribution description of the same data for *Amaranthus* in A, where the relative dose rates (R/S) on the horizontal axis are arbitrarally plotted.

C. Slow incremental increase in the dose level of resistance in repeatedly treated *Lolium* populations. The line showing how the dose required for control may increase was drawn for demonstration purposes only. *Lolium rigidum* was treated with a typical annual rate of 375g ha^{-1} diclofop-methyl. The relative dose level needed to control resistance in populations is shown as a function of the number of diclofop-methyl treatments. The sensitivity of determination of resistance was lost above a 500-fold increase in relative dose. The populations of seeds were collected in farmer-treated fields and tested by Ian Heap at the Waite Institute, Adelaide, Australia. Modified and redrawn from ref. *(11)*.

D. A population distribution description of the data in C where the dose rates on the horizontal axis are arbitrarally plotted.

In India, there is widespread resistance of *Phalaris minor* (canary grass) to the widely used herbicide isoproturon (over one half million hectares in three years since the first scattered discoveries). The first cases occurred 7-8 years after initial and then continual isoproturon applications to monoculture wheat(*24-25*). A field trip throughout the affected areas, with intensive interviews with farmers showed that resistance evolved first where farmers under-dosed the herbicide *(2)*. In a typical case the farmer initially used only half the recommended rate of isoproturon. This successfully controlled *Phalaris* for three years, but provided inadequate control in the fourth. He then used 0.75 the recommended rate successfully for two years, and unsuccessfully in the third. The full dose rate was then successful for one year but inadequate the next. Fifty percent above the recommended rate worked for a year, but no longer.

This strategy of continually increasing dose rates might be feasible for some insecticides used in high value crops, but is less feasible for fungicides and herbicides where there is far less margin between a utilizable rate and phytotoxicity to the crops. For less valuable crops, economics can also play a role in limiting the rates used for any pesticide. In North America only the manufacturer's recommended application rate of *ca* 1200 g/ha diclofop-methyl was used. In Australia such a rate was not economical in wheat cultivation and the rate of 375 g/ha was chosen because it gives adequate but hardly perfect control of *Lolium*. Thus, only a small increment of resistance was needed to change populations from susceptible to those with a modicum of resistance.

Australian conditions were conducive to rapid evolution, with increases in levels of a polygenic type resistance for the following reasons *(3)*: (a) The pest seeds were often at very high initial population densities; *Lolium* is often used as a pasture species prior to planting wheat, leaving behind fields sown with orders of magnitude more *Lolium* seed than wheat. Resistance evolved first when wheat monoculture was begun after *Lolium* pasture, and not in continuous wheat/pasture rotations; (b) *Lolium* is self-incompatible, thus different individuals with different polygenes for resistance are likely to rapidly combine. This process would be slower in a self-compatible species where most plants are pollinated with their own pollen, delaying the combination of different resistant polygenes; (c) *Lolium* produces copious amounts of pollen, facilitating the easy transfer of resistance genes by wind pollination to adjacent, herbicide-treated populations.

At the high field dose rate used in North-America, the single major target-site gene for diclofop-methyl resistance codes for a modified acetyl CoA carboxylase. It is inherited as a semi-dominant trait (*6*). The trait is functionally dominant at the rates of pesticide used in the field. A higher rate that would require homozygousity for resistance, would also kill wheat.

The Catch 22? It is necessary to understand why high dose rates preclude polygenic resistance and why low dose rates seem to delay major monogenic resistance. Such an understanding of the multiplicity of mechanisms conferring resistance is needed to design evolutionary compatible management strategies to delay or overcome the evolution of all types of resistance.

There has been much discussion by pest management specialists about which dose rates enhance the rate of evolution of resistance to pesticides, antibiotics and anti-cancer drugs. Simultaneously, theoretical geneticists have tried to deal with the first data emerging from use of these biocides, usually showing that resistance was inherited on single major genes. This is contrary to some evolutionary theory that presumed that most evolutionary change is polygenic *(26)*. This conundrum was 'solved' (*27*) with the following explanation: "Empirical data on natural and domesticated populations, and analysis of the models, suggest that strong selection sustained over several generations is usually required for adaptive evolution by a major gene mutation, to overcome deleterious pleiotropic effects generally associated with major mutations. This helps to explain why adaptive evolution by major mutations occurs much more frequently in domesticated and artificially disturbed

populations than in natural ones." One might add that the repeated selection is need to eliminate more fit susceptible individuals. Actually, the explanation may be more prosaic; in nature stresses usually appear in a gradual manner, selecting for polygenic mutants, in a similar manner as low pesticide doses, and more rarely in acute manners that are similar to high pesticide levels.

We will try to explain the Catch 22 with *Lolium* as an example, as the same pesticide selected for both types of resistance in different use patterns. In the case of *Lolium* and diclofop-methyl, genetic analysis indicates that the large target-site gene can only be mutated at a few sites under the selection pressure of herbicides, giving various cross-resistances to the two different chemical groups attacking this single target*(6)*. We will assume that 10^{-6} organisms in a pristine population have target site resistance due to one mutation per 10^6 gametes per generation. Such mutations constantly occur in the absence of pesticides. .The presence of such a mutation in a major gene can give rise to target site resistance, and in a minor gene can give rise to an increment of polygenic resistance. The frequency of such mutations do not increase in the population in the absence of selector when there is even the slightest degree of unfitness. This keeps such mutations at a steady state frequency below the mutation frequency. Without pesticide there is no selection for such mutations, precluding their accumulation. On can use as evidence for this the time it took, and number of treatments, etc., to get diclofop-methyl resistance compared to resistance to other herbicides.

The seemingly polygenic resistance to diclofop-methyl (Fig. 1C, D) is probably due to modifications in cytochrome P450s or in their levels. Because of the ubiquity of cytochrome P450 genes in families, the possibility of other mechanisms contributing to resistance*(3,28)*, and the variety of cross-resistance spectra *(11, 29)*, we can guess that there are at least 20 different polygenes and up to hundreds of genes that can each contribute to resistance. It is assumed that each polygene can independently contribute increments of resistance, and for this analysis that each contributes an average increment of resistance to ca. 50 g/ha. Clearly it is important to have real data from areas where resistance has evolved to replace these assumptions, to allow delineating strategies to delay evolution elsewhere. The possible interdependence among some polygenes, as well as chromosomal linkage groups are presently ignored. Assume here that the frequency of each resistant form of each polygene is also 10^{-6}, but we will also assume that there are potentially 100 resistant polygenes. Thus, at any time $10^{-6} \times 10^{2} = 10^{-4}$ of the pests could bear a single resistant polygene. The likelihood of any individual organism initially having two such resistant polygenes is $10^{-4} \times 10^{-4} = 10^{-8}$, with three resistant polygenes 10^{-12}, etc. Thus, there is a 100 fold greater likelihood of finding any one resistant polygene in a previously untreated population than a resistant major monogene, but a much lower possibility of finding two or more resistant polygenes. This explains how low rates can select preferentially for polygenic-inherited resistance while high rates select only for major monogenes. This has also been found under laboratory simulations for all three major groups of pesticides *(18,30,31)*. Recurrent selection at the same rate will continue to enrich for the same type of resistance in the population until resistance predominates.

Selection at a low dose rate could also select for target site resistant alleles - yet the frequency differences between 10^{-6} and 10^{-4} suggest that such an event would be relatively rare, and target-site resistance evolves slowly under low selection pressure. Indeed, years of low dose selections in Australia, resulted in the accumulation of plants containing mixtures of many polygenic, low increment contribution alleles, only recently were populations found that also contained monogenic, target site resistances *(32)*.

A pesticide dose response curve generated in the laboratory under ideal conditions is typically linear when plotted using probit techniques. This is not quite the case in the field where it is shallower and sometimes non linear; at higher doses fewer than expected organisms are killed in the field. A sprayer bouncing across a

field cannot provide the same uniform pesticide distribution pattern as a laboratory sprayer. In the laboratory pests at highly uniform age are sprayed with a highly uniform spray giving uniform distribution of pesticide and there are no pests hidden in refuges or immigrating after treatment. In the field, weeds germinate at less uniform times and two leaf and four leaf seedlings of the same species often have very different dose response curves. Some seedlings are shielded or shaded from spray by other seedlings or by clods of soil or rocks. The spray pattern is also skewed (Fig. 2). Similarly, there are often large variances in susceptibility among different insect instars, with more advanced instars being less sensitive. In the field, there is often not the synchrony achieved in the lab, and various instars are treated simultaneously. Again a skewed dose-response probit curve will be obtained. Fungi at different stages of development, germination, penetration and establishment are differently affected by fungicides. This would also cause skewing of dose response curves.

Thus, if *Lolium* is 99% controlled by 250-300 g/ha diclofop methyl in the laboratory, it takes 375 g/ha to get 90-95% control in the field (for the reasons discussed in the previous paragraph), and 1200 g/ha to get the 97-99% achieved in North America. In both cases there are some escapes due to refuges in the field, as well as late germination after the herbicide has dissipated. Presumed doses reaching different plants are depicted in Fig. 2. At 375 g/ha, the typical rate used in Australia for *Lolium*, 5-10% of the plants receive no effective amount of herbicide, and their offspring will be controlled by 375g/ha the following season if they interbreed only with each other. Another 10-20% of the population is subjected to selection for a single polygene (shaded area), because they receive 250-300 g/ha herbicide. Only a small proportion of the individuals receiving 200-250g/ha, (ca. 10^{-4}) have a polygene to allow survival, i.e., those resistant to this dose due to one resistant polygene survive. Those that survive may be severely injured but they recover.

The data in Fig. 1D depict only putatively dead/alive individuals at a fixed time after treatment under controlled conditions and thus "lose" data on sick pests that recover. After a few years of treatment of pristine populations with diclofop-methyl at low rates in Australia, there were often *Lolium* plants that appear very sick or even 'dead' Many such sick plants recovered to produce some seed (Ian Heap, personal communication). These may well be the plants with the first resistant polygene but are not yet classified as "resistant". If they could self pollinate (in *Lolium* they cannot) or are sufficiently close to another plant with the same or different resistant polygene, then 25% of their offspring would have two polygenes, and 50% one polygene. The most likely crosses by the rare individuals that survive the 250-300 g/ha treatment are with the far more ubiquitous healthy plants in the below 250 g/ha class that did not receive an effectual dose. Half the offspring from such crosses will now have one polygene. They will vastly increase the proportion of the population with one polygene the following year, and many more plants receiving 250-300 g/ha will have a modicum of resistance, spreading more pollen, increasing the chances of crosses resulting in two polygenes.

When the high dose rate of 1,200 g/ha is used, it is clear that >97% of the pests are killed (Fig. 2). Most of the survivors were in refuges and received no pesticide at all. An infinitely small proportion of plants received 250-300g, so that the selection for a single resistant polygene would be minimal. Assuming one polygene is required on average for every 50g of pesticide above 200g/ha, then 20 polygenes would be needed to survive 1,200 g/ha. There would theoretically be one plant with 20 polygenes at a frequency of $10^{-4 \times 20} = 10^{-80}$ in a pristine population. As we do not know the average increment of resistance provided by each polygene, it is better to use the statistics of polygenic inheritance: If a pristine population has a normal distribution of polygenic resistance centered at 200 g/ha and a standard deviation of 50 g/ha, then the frequency of individuals resistant to 1200 g/ha would be 20 standard deviations above the mean level of pristine resistance i.e., 10^{-88}. Either way, the only likely resistant survivors at 1200 g/ha could be those with a major gene mutant that achieves the needed level of resistance in a single step. In the case of *Lolium*, only a target site resistance seems to be a coded on a major gene. If the field is treated with a

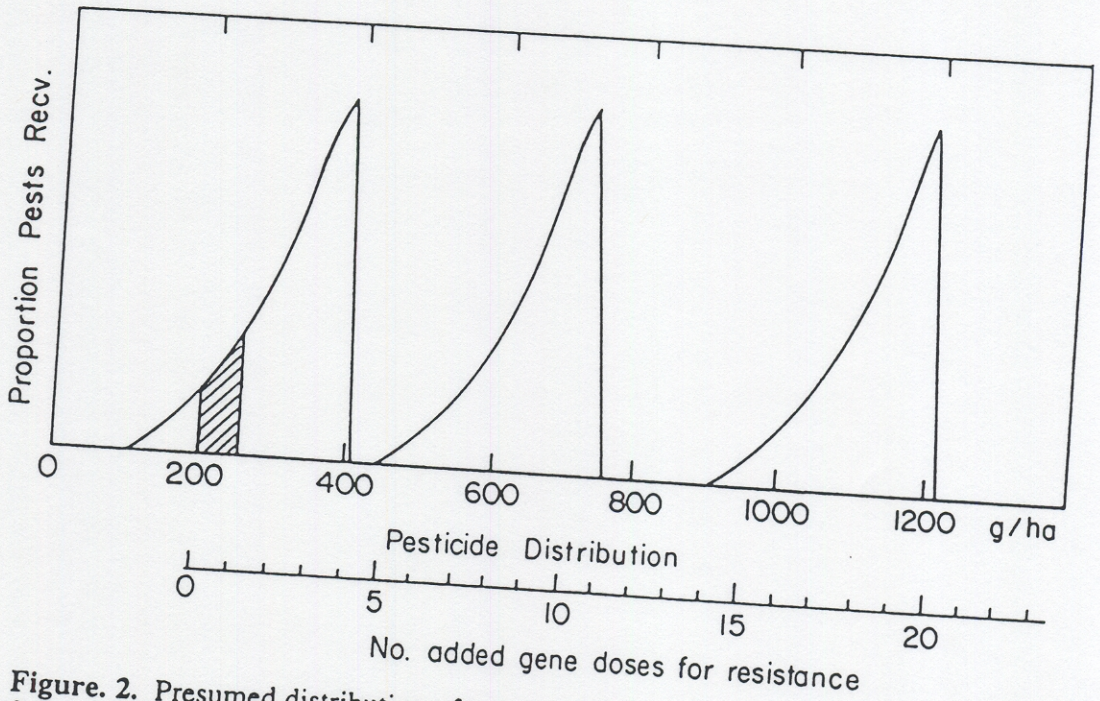

Figure. 2. Presumed distribution of a pesticide on pests following spraying in the field at 400, 750, 1200 g/ha, illustrating the proportion of pests receiving each dose. Double spraying is ignored, as are untouched escaped organisms (in "refuge"). An added scale shows how many additive polygene dosages would be required to withstand each dose assuming that each polygene provides an average protection for 50g/ha beyond the threshold of 200g/ha. The cross hatched area shows the sensitive population from which one gene dose will be selected. (Reproduced with permission from ref. *25*. Copyright 1995 American Chemical Society).

moderate dose (e.g. 700 g/ha in this case), 3-5 % of the plants receive less than a lethal dose, because they are escapees in refuges. Virtually all other plants receive a dose that would still require the combination of many rare resistant polygenes for survival. Probably, for safety sake, an intermediate dose should be chosen to require the presence of 4-8 resistant polygenes for a plant to be resistant.

Remedial Possibilities

Remedial possibilities are too often limited to an abandonment of the pesticide group in favor of others. To often pesticide salespeople have declared: "Don't worry about resistance; we always have alternatives as replacements". Thus, after losing one crop to resistant pests, a grower must often give up an inexpensive pesticide for the salesman's proffered expensive replacement. Too often, this can price the grower out of the market, and the cash crop must be abandoned along with the pesticide. Additionally, there are often cross-resistances that evolved simultaneously that further limit the choice of replacements. This seems especially problematic with the polygenic resistances that seem mostly based on metabolism. This seems to be the case in wheat, both in Australia with diclofop-methyl-resistant *Lolium* (*3,33*) and in India with *Phalaris* and isoproturon (*24*); these are cross-resistances to herbicides acting on a multitude of targets. Farmers are loathe to institute complicated preemptive resistance management schemes, especially if they cost more. Still, the best remedial strategy is to look over one's shoulder and learn from the mistakes of others. When there is resistance somewhere to a pesticide under similar cropping system, it is time to get scared, and not to say "it hasn't happened here, therefore it won't". When the first resistance appears, and it is not spread throughout the population, further enrichment of resistant individuals in the population can be delayed. The delaying tactics that have kept pyrethroid resistance at bay in Australian cotton (*34*) were instituted because it was realized that there was an incipient problem. Thailand, China, and India did not take heed, to the detriment of their cotton industries. Thus, sometimes when resistance is incipient, it is not too late to use the remedial strategy of retroactive preemptive IPM delaying tactics.

Temporary Abandonment. There are some cases where temporary abandonment can later allow for return to the pesticide with resistance. This is basically the preemptive strategy instituted when incipient pyrethroid-resistant insects were found (*34*); the pesticides at risk were used for a short period during each season of multiple sprayings with other compounds. Temporary abandonment is more problematic or even futile once resistance has become fixed throughout a population, as then total eradication of the pest must often be accomplished. Once resistance is fixed, temporary abandonment cannot work when there are internal refuges for the resistant pests to hide, or immigration of resistant individuals. Thus, it was possible, to eradicate small patches of paraquat-resistant wild barley (*35*). This weed species does not have a seed bank as a refuge; it must germinate or die the following year. As resistance was localized, there was no resistant pollen or resistant seed that could immigrate in to the fields to rejuvenate resistance. Still, it took three years to eradicate resistance using far more expensive pesticides than paraquat, and mechanical treatments to prevent seed set (*35*), and there is still a question whether there will be a relapse to resistance in those fields. Too often eradication of resistant populations is attempted after resistances has been fixed in large areas, and refuges had become full. Then it is discovered that resistance is forever.

Models predict that resistance should dissipate from the population as long as there were some susceptible individuals remaining, and there was a large fitness differential (*36*). This was not borne out by field data in one experiment to test the hypothesis (*37*). When neutral pesticides were used (those that controlled the resistant and susceptible individuals to the same extent), the frequency of resistant individuals remained constant for five years, despite the fitness differential. In too many cases

resistance is forever, and temporary abandonment is of no avail, leading to permanent abandonment of growers' most cost-effective pesticide.

Selective Abandonment of Some Pesticide Uses. There are cases where the selective abandonment of some uses has saved a pesticide for other more important uses. DDT for mosquito control is performed by coating walls and vegetation near homes with the insecticide. This allows control of mosquitoes that have recently imbibed blood and rest nearby to digest it. Such procedures exert little selection pressure on the whole mosquito population, and do not result in resistance. The widespread agricultural use of DDT surrounding villages inadvertently applied the selection pressure leading to resistance in much of the world. The only regulatory authorities to both appreciate and act on this were those of Sri Lanka, who prohibited agricultural use of DDT, and have successfully saved it for mosquito control. They alone had no problem with DDT resistance (*38*).

Synergists. Synergists in the context of resistance management are compounds that prevent the degradation of the pesticide (or its toxic products) (*39*). Such compounds can be of no avail when resistance is due to a mutation in the target-site of the pesticide. In remedial management they can suppress the causes of resistances. Inhibition of cytochrome P-450, NADPH-dependent monooxygenases are of some value already and possibly more so in the future, for managing various resistances where P450s are responsible for pesticide degradation. The P450 inhibitor piperonyl butoxide is used as part of pyrethroid resistance management (*34*), and it partially suppresses diclofop-methyl resistance in *Lolium* (*3*). As these resistances are polygenic, and many P450s are involved, and P450s are known to be differently affected by different inhibitors, cocktails of P450 inhibiting synergists may be needed to fully suppress resistance.

Synergists can also be other pesticides acting synergistically. A recent case is well worthy of mention as the implications go well beyond the initial finding. Propanil-resistant *Echinochloa* has become a scourge in rice in various countries (*9*). An acylamidase degrades propanil both in rice and the weed and it was fortuitously found that piperophos, a rice herbicide that does not effectively control either resistant or susceptible *Echinochloa*, strongly synergizes propanil on both biotypes. Surprisingly, the synergistic mixture does not affect rice. Additionally, the rates used of both in mixture, and the cost of the mixture are far less than either used alone (B. Valverde, Costa Rica, personal communication, 1995). Being less expensive, there is good reason to wonder why it was not discovered earlier, and generally used for more cost-effective pest control,. The mixture would have also been an excellent preemptive tool. Farmers lost a few season's rice crops to resistant weeds before this resistance-managing synergy was found.

The discovery groups of the chemical companies have put little emphasis on finding synergists. As cases of resistance begin to abound at greater frequency, synergists will be found to be a good way to save otherwise useful pesticides from abandonment. This should override the worry that registering a synergist costs the same as registering a new pesticide, as resistance can mean the loss of a registered pesticide. It may well be easier to find new synergists than new pesticides. Wheat should be a case in point. The Australian *Lolium* has cross resistance to all present wheat-selective herbicides, including many that had not been used before in Australia. That is probably because wheat normally uses P450s to degrade herbicides, and closely related *Lolium* seems to have evolved similar resistance as an evolutionary, biochemical mimicry (*33*).

Negative cross resistance. There have been many cases where laboratory studies have shown that some fungicides (*40*) or herbicides (*41*) control resistant biotypes at lower doses than they control the susceptible wild-type, suggesting uses both in prevention and in remediation. The remedial value was demonstrated; pyridate and bentazon selectively depleted triazine-resistant *Solanum* from maize fields (*37*) such

that there is a possibility to return (albeit temporarily) to the cheaper triazine herbicides.

Genetic Engineering. In the case of herbicides where it is hardest to find interspecific selectivities, there may be no cost-effective chemical choices, and the only hope may be genetic engineering, if total abandonment is not considered a choice. It is probable that *Lolium* will be resistant to all yet undiscovered wheat herbicides, because wheat and *Lolium* use similar P450 herbicide degradation mechanisms. Wheat needs some new herbicide resistant genes to which *Lolium* will have a hard time evolving resistance (*33*). Some such genes for herbicide degradation are available from microorganisms, e.g. to glyphosate, to glufosinate, and to dalapon (*42*), but the owners of the first gene have not made it readily available, and the second herbicide is expensive. Similar genetic engineering strategies have been used to control parasitic weeds that are normally naturally resistant to the same herbicides as their crop hosts (*43*).

Preemptive Resistance Delaying Strategies

The best strategy probably always has been to rotate crops and rotate pesticides, and it probably will remain so in the future. The vast majority of cases of resistance comes from monoculture using only one pesticide for a given target pest. Alas, you cannot rotate orchards and many crops are the only successful cash crops in a given agroecosystem. Often there are not a plethora of pesticides to chose from. Resistance management strategies must be elaborated for single pesticide monoculture, as abhorrent as it seems.

Where polygenic inheritance is involved, it has been shown time and again that the initial use of low dose rates facilitate rapid evolution of resistance, and high rates are suggested for prevention. High initial doses have also been proposed as an initial strategy in cancer chemotherapy (*44-46*), because low and then increasing doses have been shown to select for gene amplification (*23*). In the case of anti-cancer drugs, this modeling has suggested that after the first high initial doses are used, the dose can actually be dropped due to an interplay between the remaining cancer cells with the inherent immunological resistance of the patient. This could be extrapolated to agriculture, where the crop has some mechanisms to fend off small infestations of arthropods and pathogens, and can successfully compete with late-germinating weeds.

The strategy often suggested to delay monogenic resistance in monocultural, monopesticidal situations is to lower the dose rate (*36,47*). This decreases selection pressure, as a greater proportion of susceptible individuals remains after treatment, diluting and competing with the infinitesimal proportion of any selected resistant individuals in the population. There are other ways to lower the selection pressure of a pesticide where a single gene target site resistance is expected, depending on the compound and the pest situation. These include using related chemistries with less persistence, or fewer treatments with the same compound. This would allow later influxes of susceptible members oft he same pest species, diluting the proportion of resistant individuals in the population.

Another strategy often proposed is using pesticide mixtures. Too often such proposed mixtures give superior pest control but are counterproductive for resistance management (*48-49*). Indeed, criteria for successful mixtures have been delineated; they include requirements that both components: (a) control the same pest spectrum; (b) have different target sites and modes of degradation; (c) have similar persistence; (d) if possible, exert negative cross resistance or synergism with each other (*49*). The use of synergists (*39*) and negative cross resistance (*40,41*) are probably better preventive delaying tactics than they are for remedial resistance management as such strategies can be less expensive when used and farmers would not have crops lost to evolution of resistant pest populations as quickly as in the past.

Controlled Release - High to Low Dose Due to Pesticide Decay. Another compounding problem, a Catch 22 in its own right, is that when a high dose rate is used, it decays over a period of time. If there is a continuous influx of pests due to immigration of insects, spores, or pollen, or a gradual emergence from refuges (spaced out hatching, or germination of spores or of seeds) then some individuals receive a high dose and some a much lower dose. This can be addressed by repeated spraying, (which growers are now trying to decrease), or by use of controlled release formulations that release a constant pesticide dose, at a rate best attuned to delay the evolution of resistance (*50*).

Delaying Polygenic and Major Gene Resistances - Modeling

Models for Delaying Polygenic or Monogenic Resistances. We have counted more than 50 models dealing with the evolution and management of resistance in pests; and most modellers seem to believe that the pest group they work with is biologically different from all others; ignoring the rest. Most models for the evolution of resistance and its management have dealt only with major gene effects (e.g.*36,47,51-55*), and only rarely with polygenic resistance (e.g.*56*) and gene amplification (*57*). None deal with the simultaneous existence of both genetic mechanisms in the same organism, the Catch 22 situation described earlier (58).

Simultaneously Dealing with Mono and Polygenic Resistances. The model is constructed based on the following assumptions:
(A) Polygenic resistance can be delayed by preemptive treatments with low doses of pesticides with synergists, in the rare cases where available, or by treatments with moderate or high levels of pesticide. The moderate or high doses must be applied before too many resistant polygenes have accumulated due to previous treatments with low levels of pesticide; and (B) the rate of evolution of major gene resistance is a direct function of selection pressure, and low and intermediate use rates of pesticide have low selection pressures for major gene resistance (Fig. 3A). We then propose that a rotation of a number of treatments with low doses with a treatment at an intermediate dose will suppress the rate of evolution of both polygenic resistances resulting from low use rates as well as major gene resistance resulting from high use rates (Fig. 3B). The rates can only be chosen after both types of resistance have evolved somewhere, and there is evidence for the different mechanisms. The intermediate treatment is expected to control individuals that have accumulated a few resistant polygenes, setting the situation back to the initial state. The occasional use of intermediate rates might add to the cost of crop production. Still, the alternative pesticides needed when resistant populations become predominant may cost far more, if they exist. The possibility of broad cross resistances with polygenic mechanisms argues that losses will be greater if intermediate dose rates are not occasionally used. We are continually refining our first models (*25*). The modeling is based on standard quantitative population genetics for polygenic resistance and exponentially increasing monogenic resistance, and it disregards fitness differentials in polygenic-inherited resistance (S. N. Gardner, J. Gressel, and M. Mangel, submitted for publication).

This model allows for the use of less pesticide, i.e. levels that do not select only for single gene resistance, with advantages in resistance management, economy, and less environmental impact, all by lowering chemical input. The model is not to be construed as a call for stoppage of rotations of crops, cultural practices, and pesticides, which most feel provide the best possible preemptive resistance management. The model could best be used in situations where alternative crops and pesticides are impractical. Monoculture is imperative in many wheat growing areas, where land, season and/or rainfall, allow only for wheat cultivation as a cash crop, and where evolved polygenic-inherited resistance results in cross resistance to all wheat-selective herbicides.

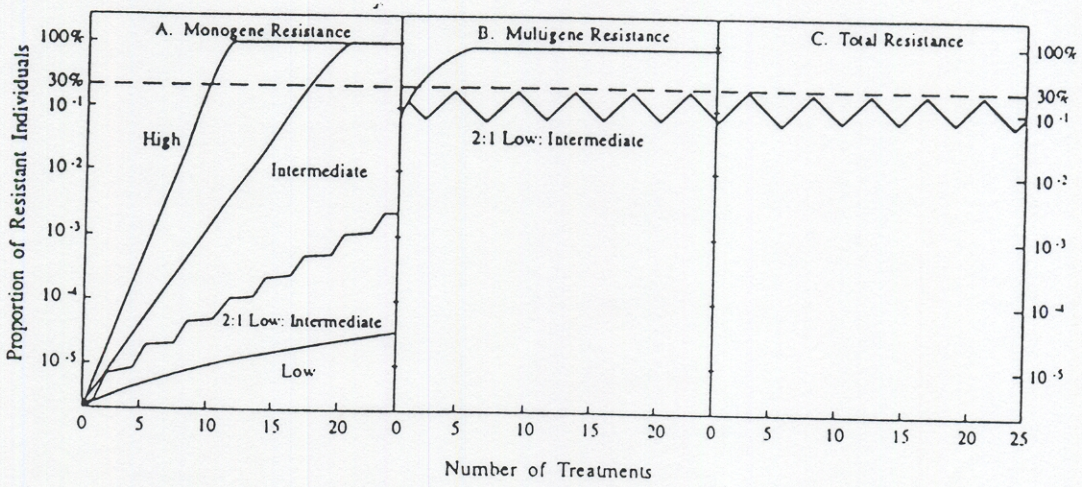

Figure 3. Effects of varying dose rates on the enrichment of different forms of resistance.

A. Enrichment for major gene, usually target site resistance. The effects of dose on the high selection pressure and medium dose with lower selection pressure (straight lines) are plotted from equations similar to those used previously (*36,47*). The calculations for the alternating doses are based on our current models.

B. Enrichment for polygenic resistance by alternating two low and one intermediate dose rates. The calculations for the alternating doses are based on our current models with immigration of susceptibles, and are based on the statistics of population genetic selections. The data are thus expressed as the proportion of the population not controlled at the rate used (see Fig. 2), which is much higher than the actual frequency of genetically-resistant individuals. An intermediate or high dose rate would (theoretically) control such a high proportion of the population that they would plot below the scale.

C. Total enrichment for all types of resistance using the alternating rates in parts A and B based on our current models.

This model has implications beyond preemptive pest resistance management in crops - it could well be considered in management schemes for antibiotic and anti-cancer drug therapies in medicine where resistance, including multi-drug resistance problems are rampant. One of the model scenarios is plotted in Fig. 3. In this scenario, it can be seen that if a high dose were used (Fig. 3A, acute slope) monogenic major gene resistance would quickly appear. If an intermediate dose alone were used, resistance would take far longer (middle straight line). A low dose would hardly select for monogenic resistance (Fig. 3A), but would facilitate evolution of polygenic resistance as shown in Fig. 1C. The application of a few low doses allows for a considerable proportion of the population (but still less than 30%) not controlled, but the intermediate dose represses the level back down (Fig. 3B).

The populaton model does not consider that the intermediate dose leaves about 5% of the population as escapees. These can genetically recombine if proximities are sufficient for breeding. The total effect such a low/intermediate rotation on both monogenic and polygenic resistances is summarized in Fig. 3C. Thus, evolution of resistant populations might occur, but at a slower rate than at the low dose alone, or high dose alone regimes. In addition to modeling, it is imperative to obtain data to further ascertain that this will happen in the field. Once resistance has evolved, e.g. with *Lolium*, one can set out experiments starting with artificially mixed populations with a few percent of resistant individuals to test management strategies, and quickly have resistant populations quickly evolve under the poorer strategies.

Such models must address four types of pest scenarios, and the outcome of resistance management strategies will vary with each. There is some overlap among them.

Type (a) scenario: no immigration and no internal refuges. This scenario fits situations of large scale agriculture where all growers are cultivating the same crop with the same pest management and where the distances are too great for more than a few susceptible pests to fly in as adult insects, pathogen spores, or weed pollen or seeds (as with the Australian *Lolium*). The internal refuges of hidden arthropods or dormant spores or weed seedbanks are very small. The selection will be the most rapid in this scenario, as there will be no influx of diluting susceptible individuals.

Type (b) scenario - where there is immigration of susceptible individuals from the outside. The outside pool is infinitely larger than the pesticide-treated group. The refuges/seedbanks are minuscule. This fits most insect and fungal cases, to a larger or lesser extent, depending on the magnitude of immigration.

Type (c). There is always a large reservoir of susceptible individuals in refuges/seed banks and there is a constant bi-directional flow between the refuges/seedbanks and the treated population. Initially, in a pristine habitat (never-treated field) the refuge/seedbank population is composed of susceptibles. This situation changes as resistant individuals enter the refuge/seedbanks, such that there is a slow, but delayed, increase in the frequency of resistant individuals emerging from the refuge/seedbanks.

Type (d) scenario - where there is both a sizable immigrant population and a sizable turnover of refuges/seedbanks.

Below we will give further examples from the type (b) situation with varying immigration and describe how resistance depends on both the fraction of immigrating susceptibles and heritability (narrow sense) of the polygenic trait. As the heritability of resistance increases, the immigrant influx required to keep resistance down below the farmer-discernible 30% level varies when a two low dose /one intermediate dose cycle is used (Table I). With the low heritability, only 7% of the treated individuals need be immigrants from the susceptible outside population. With a high heritability, 25% of the treated population must come from outside, which is hard to envisage in many cases.

Immigration has a dual effect of decreasing the rate of enrichment of both polygenic and monogenic resistances. According to the modeled findings, the effect is stronger on monogenic resistance. This allows increasing the level of the intermediate doses to set back the individuals that accumulated a polygene or two for

Table I. Requirement for Influx of Susceptible Individuals to Keep Resistance Below 30% for 50 Cycles of 2 Low and 1 Intermediate Doses

Heritability of Resistance	% Influx Per Treatment Cycle
0.3	7
0.4	10
0.5	13
0.6	17
0.7	25

resistance. In the example shown in Table II a situation is set up whereby populations with >30% resistance are kept at bay for more than fifty cycles of treatment. This includes monogenic as well as polygenic resistance. The modeled scenario in Table II requires a 33% immigration, a situation that can be envisaged with some crops and their pathogens/insects, but is hard to imagine with weeds.

Table II. Threshold Doses to Keep Population with <30% Resistant Individuals (Assuming 33% Influx per Treatment)

Heritability of Resistance	Threshold Doses (g/ha) Two Low Doses	*Then*	One Intermediate
0.3	250		460
0.4	260		480
0.5	280		500
0.6	300		530
0.7	330		562
0.8	380		620

The doses modeled here are based on the system described in Fig. 2; they would have to be modified for other pests based on what is known about the minimal effective dose with adequate control vs. high doses that have selected for monogenic resistance in the past. A similar model can be used for the cases of semi-dominant monogenic resistance, which seems common in insects (55). A low dose can be used to control susceptibles and occasional higher doses can be used to obliterate most heterozygotic resistant individuals that may have accumulated, to keep them at a low level.

The frequency of the intermediate dose can be varied, and still preclude resistance for considerable durations (Table III). When the intermediate doses are further apart, the modeled data suggest that the threshold dose must be increased, such that there is little difference in the total amount of pesticide used over a large number of cycles. It is intuitively apparent that one cannot wait too long between intermediate doses, or resistance will be over 30% by the time the grower gets around to using the higher dose. In the conditions shown in Table III, that occurs if the intermediate dose is given after the fifth treatment.

Table III. Threshold Doses to Keep R <30% at Different Dose Frequencies (Assuming 33% Influx; 0.3 Heritability)

Frequency of Intermediate	Threshold Dose (g/ha) Low	Intermediate	Total/30 doses
1:3	250	460	9,600
1:4	280	490	9,825
1:5	320	530	10,500
1:6	breaks down		

The advantage of such models is that there are easy experimental designs for testing them. Experimental verification of such models can be facilitated by mixing pristine wild type material with pests known to contain different levels of resistance. This can ascertain whether the concept of using intermediate doses after a few low doses is more than a theoretical management tool. If it is a valid tool, it must be practiced over wide areas in concert, with all but the most immobile of pests, to prevent mixing of populations allowing for enrichment of genes for resistance. In weeds, there is good evidence in some instances that each case of resistance is due to evolution within a given field, and not due to gene flow (*59*). This is not the case in insects, where flying and human transport allow for easy gene flow (*60*). There is the possibility that gene flow in mosquitoes is not as strong as proposed in (*60*), as DDT resistance has not come in to Sri Lankan populations (*38*), despite their closeness to the mainland.

Polygenic resistance seems to be potentially more dangerous to pest management than monogenic resistance, whether due to genes coding for many overlapping metabolic mechanisms, or to amplifications resulting in multi-drug resistance. This is because resistance can be to a large spectrum of chemicals with different modes of action. Thus, we must weigh the risks of each alternative Catch 22 situation. Many more cases of polygenic resistance should be expected as more farmers cut doses to adhere to strictures to lower pesticide levels. It may well be wise to consider using no pesticide when infestations are low instead of using a low dose, unless the low doses are interspersed with higher doses.

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