

Problems in qualifying and quantifying assumptions in plant protection models: Resultant simulations can be mistaken by a factor of million[☆]

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Abstract

Models related to plant protection can give an appreciation of a phenomenon, and provide ideas for priorities in research, as well as suggest management strategies. The problem with models is that mistakes can be huge when inaccurate assumptions are made about key parameters, as described with three sets of models: (1) our own model predicting that five herbicide-resistant *Striga* plants would appear per hectare per season was based on an inaccurate assumption that heterozygotes would be selected, and a heterozygous mutation frequency was used, while a recessive mutant frequency should have been used. A revised model with a recessive mutation would predict five resistant plants per million hectares per season; (2) the model predicting that Bt resistant insects would quickly evolve in transgenic cotton and maize unless massive refuges were instituted, assuming a single binding site for the toxin and minor unfitness of resistant individuals, not realizing that resistant individuals may be extremely unfit or that Bt may have multiple targets; (3) a model that claims that unfit transgenes from crops would decimate wild relatives by swamping. The model assumed animal-type low progeny numbers, and did not consider competition for replacement, nor the infrequency that crop pollen could reach wild relatives. Models must be retrospectively critiqued, based on field data and new knowledge, and not be allowed to become accepted as being axiomatic.

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1. Introduction

Models of plant protection, whether dealing with pesticide resistance, or issues of gene flow from transgenics have been very useful in pinpointing critical issues for dealing with problems in plant protection. Good models can help delineate priorities to decide which parameters are most likely to affect a phenomenon, and suggest which research should first be performed to ascertain correctness so that better plant protection strategies can be elucidated. Many of the

models have been verified by either experimentation or by large-scale epidemiological data. Some have not. The problem is that models are often used for recommendations to growers before the underlining assumptions are verified, sometimes at great risk or cost to companies and/or the growers. For example, untested models that we developed (Gressel and Segel, 1978, 1990) were used by manufacturers of highly persistent (high selection pressure) herbicides to recommend use every other year, temporarily costing half their sales, but probably much more than doubling the useful life of the herbicides in question. Resistance was rampant where the strategies were not used, and successfully delayed where there was compliance. Conversely, there is evidence that the regulatory requirements for 20% refuges when growing transgenic Bt cotton and maize, based on models, were superfluous. Additionally, a model has been published

[☆]Dedicated to the memory of recently deceased mathematician, Prof. Lee A. Segel, a collaborator in herbicide resistance modeling for nearly three decades, who continuously warned this author while working together: “garbage in, garbage out”. How right he was.

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predicting that unfit transgenes flowing from crops to related wild species will swamp wild populations and cause them to go extinct (Haygood et al., 2003), which remains to be questioned. These models have all appeared logical to peer reviewers, to journal editors, and to readers, etc., yet they can be inaccurate, despite the consensus, because they inaccurately qualified or quantified parameters in the models.

There has been little published analysis comparing actual outcomes with the predicted outcomes of models, which is unfortunate, as it does not provide the warnings to the next generation of modelers as to what might go wrong when model assumptions are inaccurate, or key parameters are missing. Models are often assayed for correctness by sensitivity analysis, but too often these analyses are based on the faulty assumptions underlying the models.

Field epidemiology demonstrated that a model we made (Gressel et al., 1996) had to be wrong. The model predicted that five individuals of the parasitic weed *Striga hermonthica*, resistant to acetolactate synthase (ALS) inhibiting herbicides would appear per season in maize, yet none appeared on hundreds of hectares. We were slow to realize that we had experimental data that explained why we were wrong. We describe below at some length why this is the case, and describe two other models that also seem to be questionable, pinpointing where inappropriate parameters, values, and/or assumptions were probably used when the models were formulated. In some cases, early experimentation could have helped refine the models, in other cases this would not have been easy.

2. Case studies

2.1. Model for evolution of *Striga* resistance in imidazolinone-resistant maize—off by a million?

S. hermonthica is a major pest of grain crops in Africa, especially the staples, maize and sorghum, infesting the lands of a 100 million Africans (Gressel et al., 2004), lowering yields by half, rendering fertilizer use uneconomical, causing land abandonment, and population displacements.

A decade ago, we demonstrated that such parasitic weeds could be controlled in crops that had transgenic (Joel et al., 1995) or mutant (Abayo et al., 1998) target site resistance to systemic herbicides. Imidazolinone resistant (IR) maize, bearing a mutant ALS resistance was used with seed treatments of the herbicides imazapyr and pyriithobac to successfully prove the concept. While the gene was backcrossed from US maize to elite African lines, and the seed coating technology perfected (Kanampiu et al., 2001, 2002, 2003) we modeled the expected evolution of herbicide-resistant

Striga populations (Gressel et al., 1996), to ascertain what precautions might be taken. The model predicted that there would be five new resistant individuals appearing per season, and predicted that fields would be covered with resistant individuals in 10–12 seasons when such seed treatments with herbicides were used. The model considered the known dispersal distances of *Striga* seed in a situation where maize is hand harvested (machinery would disseminate weed seed more quickly over larger areas), the known seedbank longevity of *Striga*, estimated attachment rates based on observations, seedbank size from others' studies, a very high kill rate of *Striga* (as evidenced from our preliminary observations with the technology), and the mutation frequency of resistance in other weeds that evolved resistance to ALS-inhibiting herbicides, data from both mutation selection experiments and from the field, of about one resistant plant per million treated (Darmency, 1994; Saari et al., 1994; Gressel, 2002).

The model indicated that if the technology was to be sustainable, farmers must scout their field and rogue these five resistant individuals per hectare before the resistant plants set seed. Of course the technology would last an infinite period if all resistant individuals were rogued, and even if only four were removed, the technology should be good over 20 seasons, according to the model.

In the mean time, the trait was backcrossed into elite African maize and the technology optimized (Kanampiu et al., 2003). At various stages during backcrossing, material from both homozygous open pollinated synthetic varieties as well as hybrids was made available for extensive testing in farmers' fields and experiment station sites. In nary a field was an early emerging, robust *Striga* sighted, the type that would indicate resistance. By the time hundreds of hectares had been treated, we started questioning the model. Where were the resistant individuals and populations that have been predicted? Simple statistics state that there was a 95% certainty of seeing at least one resistant individual after only a few hectares were treated if five resistant plants were expected.

In analyzing the parameters we finally remembered that during the backcrossing, the breeders had to be careful to use much lower herbicide rates to chemically cull the 50% of the plants that were susceptible, so as not to kill the other 50% that were heterozygotes. The rates of herbicide being used on the maize seed to control *Striga* required that the maize have homozygous resistance. The rates typically used in field in other agroecosystems, where the herbicide is evenly spread by spraying, selects for resistant weeds, and these individuals are heterozygous. The mutation frequency of 10^{-6} in the model is based on the frequency of heterozygotes. The highly localized concentration of herbicide coming off the maize seed would kill a heterozygous mutant,

only a homozygous mutant could survive. These would exist at a frequency of $10^{-6} \times 10^{-6} = 10^{-12}$. This new assumption would model out as five resistant individuals per million hectares per season, and not the originally predicted five per hectare (Gressel et al., 1996), a result suggesting that the risk of resistance is lowered a million fold. This indeed may be the case in Kenya, where a 12–14 week season maize is cultivated. The few *Striga* stalks that do emerge late in the season (10–12 weeks) indicate that there was some dissipation of the herbicide, but these late emerging plants do not set seed in the short season.

In some parts of Africa 18–22-week season maize is cultivated. There is a possibility that the herbicide will sufficiently dissipate, allowing heterozygous resistant individuals to emerge and set seed. In such areas there may indeed be up to 5 resistant *Striga* appearing late in the season, needing to be rogued, as per the original model. Clearly, there will be a need to monitor for resistance in those areas, while places with shorter season maize require less intensive monitoring. Slow release herbicide formulations are being developed for the areas with longer season maize. With such formulations, it may be possible to keep a high enough local concentration, long enough into the season, to allow only homozygous recessive mutations to exist.

The “advantages” of a weed such as *Striga*, with its huge seed output (up to 10^5 seeds per stalk, with multiple stalks parasitizing single maize plants), is that relatively small experiments, on tens of hectares allows elucidation of whether resistance evolves according to a model, with or without slow release formulations. Whatever the outcome, it should be easier to obtain compliance vis-a-vis herbicide rate and formulation if we can demonstrate that the model is correct and such strategies are necessary.

2.2. Bt—why no insect resistance in transgenic maize and cotton despite widespread use?

The advent of transgenic crops engineered to produce the insecticidal protein toxins of *Bacillus thuringiensis* (Bt) brought with it the expectation of rapid evolution of resistant insects. The diamondback moth had already evolved resistance to direct applications of the Bt bacteria in organic agriculture (Tabashnik et al., 1990), which assured specialists that resistance would evolve to Bt in cotton and maize. Later, cabbage loopers evolved resistance to Bt sprays in greenhouses (Janmaat and Myers, 2003). One of the arguments against deployment of transgenic Bt resistant crops was that their widespread use would engender rapid evolution of Bt resistance, jeopardizing the effective use of Bt sprays in organic agriculture. If anything the opposite has occurred. Far greater areas of Bt transgenic crops were planted than the small areas where the bacteria had been

used as a spray. The transgenic crops had a much higher toxin concentration, with greater persistence than when the bacteria are used. Models predicted that with this greater selection pressure, resistance should quickly appear. As resistance seemed inevitable, further models were developed, based on high dose rates and leaving 20% of the total area planted in separate refuges, that if followed, were expected to delay the first discernable cases of resistant populations to 5 years from the beginning of widespread cultivation of Bt crops. The models included the standard items; mutation frequency to resistance, selection pressure, expected fitness of resistant individuals and pinpointed the mobility of insects as a parameter that could be modulated to delay the appearance of resistant populations; by requiring the possibility of continuous fresh influxes of susceptible individuals from areas surrounding where Bt transgenic crops were cultivated to dilute resistance with slightly more fit individuals (Frutos et al., 1999; Carriere et al., 2001; Tabashnik et al., 2003, 2004; Caprio et al., 2004; Sisterson et al., 2004). As a high dose is used, it was hypothesized that resistant individuals would have to be homozygous recessive, and the influx of susceptible individuals from the refuges would delay the inevitable cross between two heterozygotes. The models suggested that only if 20% of the crop was non-transgenic, planted in a block near the transgenics, would there be enough immigration to delay massive resistance for the stipulated 5 years when the initial varieties could be replaced by varieties with stacked (pyramided) genes, where the frequency of resistant individuals could be lowered. Five years have long past, resistant populations are not rampant where the farmers placed refuges, as modeled. Typically, modelers start their models by justifications such as: “An adaptive management strategy can be used to hedge against failures in the scientific assumptions underlying the high-dose plus refuge strategy for managing resistance.... This will require sensitive monitoring coupled with management interventions” (Andow and Ives, 2002), but say nothing about obtaining genetic, ecological and biochemical data to plug in to the simulations to justify the model.

Conversely, Vacher et al. (2003), modeled that low doses would be preferable, which was hotly debated by Tabashnik et al. (2004), claiming that the other group was using invalid assumptions about larval responses to Bt dose as well as the dominance of resistance. Most models assume that resistance is recessive and only one locus is involved (Tabashnik et al., 2000, 2004; Ives and Andow, 2002; Storer et al., 2003; Cerda and Paoletti, 2004), an assumption that is not backed by data, as resistance has yet to evolve in the field in maize or cotton. Recurrent selection by leaf feeding in the laboratory for 12 generations resulted in populations that had a modicum of resistance and after 45 generations, resistance to exogenous toxin increased

1000-fold (Meng et al., 2004), but we know nothing about the genetics of these resistant individuals, nor whether they are fit and can compete in the field. Bourguet et al. (2003) following field screening for resistance posit that others' estimates of the frequency of resistance alleles were too high. Is that the reason resistance has not yet evolved? Then why could resistance be selected for by recurrent selection on small populations in the laboratory? The same group had warned 5 years ago (Bourguet et al., 2000) that the recessiveness is not the most important factor—one must know the fitness. Was anyone listening?

The modelers now proclaim that the modeled strategy worked even better than predicted. This is fatuous, as more than 80 million ha of Bt maize and cotton have been planted worldwide since the inception of transgenic Bt crops, nearly a decade ago, with and more than 12 million annually for the last 5 years. There are no resistant populations plaguing the fields, as had been predicted. A considerable amount of that was in monoculture cotton, where the selection pressure is high. No resistance has evolved in cotton and maize where farmers did not comply with the modeled recommendations for refuges, where resistant populations were expected to appear in just a few years. This includes 20% of US farmers who did not comply, according to surveys (see excellent perceptive review by Bates et al., 2005). There are no requirements for refuges in China, growing nearly 3 million of hectares of Bt cotton annually. If there are partially resistant individuals out there, as claimed (Andow et al., 2000; Bentur et al., 2000; Tabashnik et al., 2000), certainly two such individuals would have mated by now giving rise to 25% homozygous resistant individuals.

Where are the Bt models wrong? As there is no real field resistance in cotton or maize, it is hard to ascertain. Two major possibilities emerge, which separately or together may be the cause that resistance is not rampant, the assumptions that: (1) there is a single target; and (2) that resistant individuals would be rather fit. These possibilities are discussed below.

2.2.1. Is there a single binding site—implications to frequency of resistance

Bt can affect more than one target site on insect intestinal mucosa. There is considerable evidence that Bt Cry 1Ac used in the transgenic maize and cotton binds to both a cadherin protein (e.g. (Flannagan et al., 2005)) and to an aminopeptidase N (e.g. (Bravo et al., 2004)) in European corn-borer (*Ostrinia*) gut membranes, albeit with different affinities to the toxins and to different oligomerization species of the Bt Cry protein. It seems that the Bt levels in transgenic cotton and maize are high enough in the transgenic plants to affect both sites. Multisite binding was also shown for CryIc toxin on *Spodoptera* gut membranes (Avisar et al., 2004).

If two different proteins bind, it is highly likely that either could mutate to non-binding. If that is the case, more than one gene would be required to mutate—and the present models all assume a single gene mutation, at a much higher frequency (10^{-3}) than is experienced with mutations in non-insects $<10^{-6}$. The immunological studies with the cadherin receptor in European corn-borers suggest a family of cadherins—even more genes that would have to mutate—and if the modelers are correct in their biochemical assumptions—that high-level resistance is recessive, the resistance would be at an exceedingly low frequency. Low-level resistance seems to be controlled by an incompletely dominant gene controlling lowered synthesis of a midgut protease that is required to activate the Bt toxin, further complicating the genetics. As the modeled guess was 10^{-3} for a heterozygous mutation frequency, homozygous resistant individuals would be expected at a frequency of 10^{-6} . Surely in the millions of hectares treated, two such individuals must have met to mate. Intuitively, the frequency of 10^{-3} seems misconceived. In microorganisms and plants natural mutation frequencies are closer to 10^{-6} , and if this were correct for insects, the models could be off target by a factor of a thousand. If two genes must be mutated, and be in recessive form, then the models would be off by millions.

One might posit that there is but a single target site receptor in the few insects that evolved resistance to Bt sprays. Indeed, Bt resistance in the cabbage looper is inherited as a single incompletely recessive gene (Kain et al., 2004). The scientists developing Bt strategies for maize in Africa, where there are three different stem-borers, with different susceptibilities to different Bt toxins, are using binding site information to design the optimal mixture of genes that will assure that a few targets on each insect will be affected, far lowering the risk of evolution of target site resistance (Rang et al., 2004). Clearly, the homework is being done better for Africa, to have multiple sites affected, than seems to be the cases with the diamondback moth and the cabbage looper, which seem to have single targets for each Bt toxin.

2.2.2. Are resistant individuals fit

Perhaps the unfitness of resistant mutations is so high at high levels of Bt that resistance is effectively lethal in nature, especially the compounded unfitness of mutations at more than one gene. Unfortunately, the entomologists performing the few fitness experiments with low-level resistance populations that have evolved provide misleading evidence to those that use the term fitness based on Haldane's (1960) definition; the ability to reproduce compared to the wild type in the absence of the selector (Bt in this case). What has been called fitness in the papers on Bt resistance is in reality “productivity”—the comparable ability to grow and survive when

cultured separately, often without even measuring fecundity. There are many examples of resistant and susceptible organisms having near equal productivity and fecundity when grown separately, but in competition between resistant and wild type, the resistant individuals disappear. This is almost axiomatic that this should be the case, if resistant mutations were fit, i.e. the mutation neutral, or very near neutral, there would be pockets where there are resistant populations (Sewell Wright drift, in the parlance of the evolutionists). (The fact that many of the entomologists consider the appearance of resistant individuals and populations as a “developmental” process, as that is what they call it, instead of an evolutionary process, may affect their outlook and understanding.)

Fitness experiments must be done in the wild, with the resistant individuals in competition with the wild type, preferably in a replacement series (De Wit, 1960) and not in the laboratory on artificial media, where perhaps all components of a midgut may not be needed. Perhaps leaves have other compounds that further reduce the fitness of resistant individuals, or there are environmental conditions in the field affecting fitness not mimicked in the laboratory. Indirect experiments indicate that indeed there are fitness differences—recurrent selection for 9 generations raised resistance levels to 160 times the original, (in a gradual whole population manner, suggesting quantitative inheritance), and resistance gradually disappeared at the same rate, when taken off Bt (Bolin et al., 1999), suggesting that resistant individuals were less fit.

2.2.3. Were refuges necessary?

Conversely, one may question whether it was advisable to go to market with draconian refuge requirements, or go to the lab and answer basic questions (as is being done for African maize stem-borers) so that more knowledge-based models could be achieved. The refuges cost the farmers that adhered to them a loss of yield and an increase in production costs, that non-complying growers did not sustain. The number of target receptor proteins—implicating the number of genes, could have been quickly elucidated long ago with a concerted effort. Indeed, when the various targets and cross resistances were elucidated with the diamondback moth (Zhao et al., 2001), it was possible to construct a crop containing stacked Bt genes, which killed the individuals that were resistant to this or that Bt toxin (Cao et al., 2002). The fitness of resistant individuals can only be ascertained after there are true target site resistant populations in a maize or cotton field, a Catch 22. Clearly, the lack of resistance on the large areas where resistance management practices were not instituted negates the statement that “the development, deployment and regulation of insect resistance management strategies that are firmly rooted in theory

and supported by experimentation” (Bates et al., 2005) contributed to the lack of evolution of resistance.

The reasons why Bt resistance has not evolved in maize and cotton, and when it will evolve are quite a mystery, and unfortunately, models will probably not help until we have resistance in these crops, and know the correct parameters and values to insert into models. Unfortunately, those concerned with Bt management were worried about “repeating the pesticide treadmill that has dominated pest management for the past five decades, where products were used until resistance causes them to fail” (Bates et al., 2005). The models they built were based on assumptions derived from knowledge of the single target insecticides of the past five decades. Perhaps, from a resistance point of view (but thankfully not from a toxicological point of view), Bt is like the insecticides from 6 decades ago, the cyanides, and arsenicals, which have multiple targets and were widely used for more than half a century, and were far less prone to the evolution of resistance than the second generation of synthetic organic insecticides that replaced them. Nature may have designed Bt to be resilient against the pests of cotton and maize by multi-targeting. Nature uses synergies far more than the single, stand alone solutions desired by the crop protection industry.

2.3. A model of genetic swamping of wild species by crop transgenes

A widely acclaimed (in news releases) recent model claims that ‘demographic swamping’ by crop transgenes would cause ‘migrational meltdown’ of wild species related to the crop, especially if the introgressed genes confer unfitness (Haygood et al., 2003). This proposition that recurrent gene flow from crops, even unfit gene flow, could affect wild relatives deserves some discussion, as it flies in the face of Darwinian concepts of survival of the fittest.

Data with conventional crops already belie this possibility that recurrent gene flow from transgenic crops with less fit genes will cause wild populations to shrink. Major domesticated crops are not fit to compete and live in wild ecosystems, so their normal genes should confer a modicum of unfitness. Crop x wild hybrids continually form at a low frequency, yet there is no published evidence that demographic swamping has occurred from recurrent gene flow from conventional crops, and the authors supply no data to support their model simulations. Indeed, considerable evidence has been presented in many crops exist near their wild or weedy progenitors, without causing the extinction of the progenitors, despite continuous gene flow (Gressel, 2005).

There are other flaws in their model that are based on questionable premises and assumptions, not borne out

by plant biology. Three problematic issues that seem to invalidate their model for the vast majority of conceivable crop/wild species systems, are discussed below:

(1) To get the level of swamping that they discuss (Haygood et al., 2003), the wild relative and the crop would have to live in the same ecosystem. There is typically geographic separation between agroecosystems and wild ecosystems, with pollen flow decreasing exponentially with distance—usually to a low asymptote due to wind currents or insects, not fully following simple physics. There should always be far more wild pollen in the wild ecosystems, so hybridization events in the wild from crop pollen will be rare, even with masses of pollen occurring within the agroecosystem. Thus, their basic assumption of crop pollen swamping wild type pollen in the wild, is most probably invalid. Indeed, even when they assume an enormous 10% of hybridizations in the wild each generation coming from crop pollen, according to their model it will take about 20 generations of recurrent pollination for the unfit crop allele to become fixed in half the population (Fig. 1a), and 50 generations for a unfit gene to asymptotically reach 80% of the population. The model is contradicted by experiments (Al-Ahmad et al., 2005) where a replacement series was used and a transgenic crop bearing an unfitness gene was competitively intermingled with the wild type and progeny counted. When 9 times more unfit individuals swamped fit individuals, the result was less

than 90% unfit progeny, and simple extrapolation demonstrates that every year with recurrent selection the unfit genes will gradually disappear (Fig 1b).

(2) They assume synchronous flowering, no self-fertilization, and no genetic or other barriers to cross-fertilization; indeed, this negates the definition of speciation. It is exceedingly rare for crop pollen to fertilize another species without any genetic barrier in the wild relative. They suggest no cases where this might happen, but in reviewing the literature on interbreeding wild relatives of crops one sees that it might only occur with con-specific wild sunflowers, which might fit this criterion, but even in this case there are genomic deterrents to introgression (reviewed in Stewart et al., 2003). Con-specific domestic rice and red (weedy)-rice does not fit their assumptions because they are cleistogamous, predominantly self-fertilizing before the flowers open, and the amount of outcrossing possible would be very low. Of course weedy rice is not a wild species (by definition), so it too is not really relevant to their case. There are fertilization barriers of different chromosome numbers, non-homology etc, which limit fertilization of wild relatives by crops of oilseed rape and wheat, so they are outside the model stipulations.

(3) Their model assumes animal-type replacement rates where just a few progeny per mating is typical, allowing lower fitness to indeed become fixed. Most wild relatives of crops produce copious amounts of seed to replace parents. Hundreds to thousands

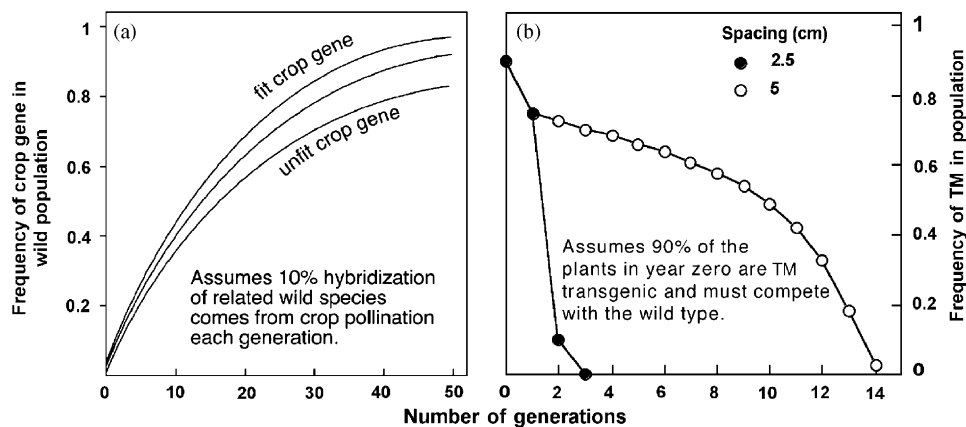


Fig. 1. Will unfit transgenic hybrid plants establish? Two views; modeling vs. experimentation: (a) Modeling of gene introgression under recurrent gene flow from crops to wild relatives by Haygood et al. (2003). If validated, it would preclude the use of transgenes that are positive or neutral for crop but unfit or a wild or weedy species linked to the gene of choice (transgenic mitigation) (Gressel, 1999) as a failsafe mechanism to prevent transgene establishment in volunteer weeds and related weeds in agroecosystems. Note that the model starts with 10% introgression per year. Source: redrawn and annotated from Haygood et al. (2003). (b) Elimination of the transgenically mitigated plants from the population under competition with wild type, starting with a swamping 90% of the population as 2:1 hemizygous to homozygous transgenically mitigated plants that hybridized with the wild type and were segregating into the wild type population in season zero. The mitigating unfitness gene being used is the truncated *gai* (gibberellic acid insensitive) dwarfing gene that increases the reproductive rate (harvest index) of the crop (Al-Ahmad et al., 2004). The data points are based on flowers formed per season per unit area of the TM and the wild type plants grown together in a replacement series at close spacing, as described in Al-Ahmad et al. (2005). The data from the results in season 1 were used as the starting frequency for the next season, further using interpolated data from the replacement series to calculate the results for the following season. This figure was originally published in Al-Ahmad et al. (2005), and is reproduced by permission of Springer Verlag.

typically germinate in the area occupied by a parent and the process of self-thinning is ferociously competitive, eliminating less fit individuals. Self-thinning, except by sperm during fertilization, is far less an important factor in animals than plants due to the low progeny number, as well as the fact that most animals are “perennial” and most wild relatives of crops reproduce a single generation in their adult life.

Their conclusion that “the most striking implication of this model is the possibility of thresholds and hysteresis, such that a small increase in (unfit gene) immigration can lead to fixation of a disfavored crop allele....” (Haygood et al., 2003) flies in the face of evolutionary evidence, and decades of classic and contemporary field data showing that only near-neutral genes exist in pockets of the evolutionary landscape of plants, and blatantly unfit plant genes are not known to exist in such pockets unless all the fit genes are somehow removed. Just as endogenous unfavored gene mutations exist in the wild at a frequency lower than the mutation rate, crop transgenes that have a fitness penalty will exist in the wild at a rate lower than the immigration rate. As discussed above, the immigration rate to the wild is perforce very low. Unfit genes are eliminated from populations of plants that produce large numbers of seeds, whereas they could be fixed in populations of animals with few progeny. When a model contradicts reams of data, it is more likely than not that the model is invalid.

Haygood et al. (2003) further contend that their model would work if the crop were heterozygous for the unfit gene (and many hybrid crops have the transgene in a single parent and are thus hemizygous). The data in Fig. 1b clearly show that when even 90% of the starting population contains a hemizygous unfit gene, these plants cannot compete with their non-transgenic sibs, let alone the wild type. Part of the problem may be that Haygood et al. (2003) (p. 1880 column 2) “assume (that) the number of plants surviving to maturity does not vary from one generation to the next”, a questionable assumption for unfit phenotypes when they must compete with fit cohorts and other species.

Where might their model have some validity? Even though, despite their claims, the model has limited validity for the ‘wild’ ecosystems, the model might be valid for a few weeds (not wild species) related to crops. When flowering weeds are at a low density in an agricultural ecosystem (and perhaps close by in ruderal systems) the model might be predictive, but would this be so bad to see a weed go extinct? As weeds are (inadvertently) man-made domesticated species (Warwick and Stewart, 2005) should not people also have the right to eliminate them? The nature of weeds is such that they do not go extinct, as much as the farmer would

desire. It is far more likely that such evolutionarily threatened weeds would evolve exclusionary mechanisms that would block evolutionarily threatening gene flow, e.g., they would evolve a shift to predominant self-fertilization that would protect them from crop pollen bearing unfit genes.

3. Discussion

This author can only exclaim *mea culpa* for the misconceived model he co-authored (Gressel et al., 1996). The data show that one should not be complacent in modeling the future, based on assumptions that may have been correct based on past experience. ALS herbicide resistance in the field had always been at a mutation frequency of ca. 10^{-6} , why expect 10^{-12} ? We have to continually give nature credit of knowing more than we do. In the case of *Striga* our mistake comes out best for the farmer.

So far the Bt modelers emit gurgles of surprise that resistance has not appeared, crediting their models as being better than they thought while forgetting the vast areas of non-compliance, which demonstrated that the needs for refuges were not as the models predicted. They claim that “the high dose/refuge strategy is still considered by most experts to be the most effective strategy currently available for delaying resistance” (Bates et al., 2005). The paucity of evidence to support that claim illustrates the need for a lack of democracy in science. From a genetic point of view, stacking genes is probably the best strategy, as the frequency of resistance alleles becomes compounded, and if the targets and genes are sufficiently different, the frequency can become infinitesimally low—until nature evolves a non-target, multiple resistance mechanism, such as multiple drug resistance, or a single pathway for toxin degradation. Thus, stacking Bt with non-bacterial toxins, e.g. the highly specific spider or scorpion venom genes (Nakagawa et al., 1997; Lorent and Hammock, 2001), or synergizing against resistance using endochitinase genes, which by themselves have no effects (Regev et al., 1996) might provide a longer life, than the multiple Bt’s, (mixtures of Bt Cry proteins as well as Bt Vip proteins, discussed by Bates et al., 2005) by going to mixtures of mechanisms that are less likely to be found in nature.

The models for genetic swamping seem not to be scientifically motivated, based on the polemics of their discussion, the generalities of the phenomenon they expect, and the lack of the authors suggesting a single crop/wild species agronomic situation, where they think that the swamping they suggest could occur. In their case, none of the assumptions are based on real case data with plants. Those models demonstrate beautifully

why one should not extrapolate from models developed for low fecundity animals to prolific plants.

In conclusion, Nobel laureate Manfred Eigen wisely stated: “A hypothesis has two possibilities, it can be right or wrong; a model has a third alternative, it can be right, but irrelevant”. Some models have a fourth alternative, they can be wrong, misleading and irrelevant. The moral to a fable by James Thurber is also quite applicable to modeling: “get it right or let it alone, the conclusion you jump to, may be your own” (Thurber, 1956). In reading models, never start with the bottom line; check the assumptions. When the authors of a model declaim “the model proves”, it is advisable to immediately discount the model. Models can and have been useful in pest management, but, as with hypotheses, the best way to demonstrate utility of a model is to perform experiments to invalidate it. One should be quick to abandon models that do not meet field data—a model cannot be “better than expected”.

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