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A revolving dose strategy to delay the evolution of both quantitative vs major monogene resistances to pesticides and drugs

(*Keywords:* pesticide resistance, drug resistance, evolution of quantitative characters, monogene resistance, dose strategies)

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Abstract. The evolution of pesticide and drug resistance presents a daunting problem as the pests often evolve resistance after a short time. Debate once focused on whether resistance stems primarily from single major gene sources or sequential accumulation of quantitative traits. Such debates became moot, as examples of both are known, even within the same species and population. The type of resistance has important implications for determining the dosages that will delay the appearance of resistant populations. Successive high doses of a toxin (i.e. those well above the LD_{99}) can only select for monogenic resistance, while the weaker selection pressure of low (near lethal) doses can also result in an incremental increase in quantitative changes leading to increasingly higher levels of resistance. We propose and use a model to mathematically test whether a revolving rotation of a series of low doses periodically alternated with a higher dose could delay the evolution of resistance longer than by applying a constant dose (either low or high) in every treatment. We suggest this dosage rotation strategy for situations in which crops and alternative pesticides or drugs cannot be rotated, and suggest that it be used in conjunction with other methods of control (biocontrol, IPM) to further delay resistance. We model the evolution of resistance under truncation selection (more typical in the laboratory), as well as under a smooth selection regime that is more likely to describe selection in the field where pests develop more asynchronously, and application is less uniform. In addition, we consider the level of population control imposed by the toxin, since, for economic and health reasons, both the size of the pest population as well as the frequency of resistant pests must be considered. We also examine how the degree of dominance of the major resistance gene and changes in heritability with repeated episodes of selection affect the success of dosage rotation compared with constant doses. Modelled dosage rotations could delay resistance and suppress pest populations longer and require less pesticide (or drug) than successive constant doses. Constant doses may suffice to delay resistance over the short run if heritability is low, but revolving doses are essential over longer periods. The modelled results seem worthy of field validation, as they predict a longer resistance-free period at the lowest possible doses.

1. Introduction

The evolution of resistance to pesticides (Brown, 1996; Powles and Holtum, 1994), antibiotics (Baquero and Blazquez, 1997), and chemotherapy drugs (Goldie and Coldman, 1985; Stankovic *et al.*, 1992; Panetta, 1998) presents an escalating problem worldwide. All too often, after only a few successive treatments, pesticides or drugs no longer effectively kill infesting organisms or cancerous cells due to the evolution of resistance. This is an especially troubling situation if no alternative pesticides or drugs exist, or if there are broad cross or multidrug resistances. There has been some discussions as to how the strength of selection imposed by different dose rates affects the rate of evolution of resistance (Gressel and Segel, 1978; Lande, 1983; Via, 1986; Coldman and Goldie, 1987; Birch and Shaw, 1997). Geneticists have shown that resistance can result from both major gene or quantitative mechanisms (Orr and Coyne, 1992; McKenzie and Batterham, 1994, 1995; Groeters, 1995; Tabashnik, 1995).

The particular genetic mechanism controlling a given resistance has important implications for the choice of dosage strategy most likely to be successful in delaying resistance. Resistance conferred by many incremental changes, each with a small effect, whether by many different (poly)genes, by gene amplification, or by sequential mutations within a gene, each increasing resistance (hereafter grouped under the term 'quantitative resistance') appears gradually after selection with low or incrementally increasing doses (polygenic, Hollomon, 1981; Via, 1986; Shaw 1989; amplification, Rath *et al.*, 1984; Roninson *et al.*, 1984; Schimke, 1984; Caretto *et al.*, 1994; Melguizo *et al.*, 1994; sequential mutations in same gene, Choi *et al.*, 1988).

Farmers are under extreme economic and environmentalist pressures to reduce pesticide usage. Similarly, the medical profession is under pressure to reduce antibiotic usage. One way to do this is to use lower doses, and more cases of quantitative resistance are to be expected (Gressel, 1995a). As will be discussed in detail, high doses applied from the start prevent quantitative resistance from accumulating because it is highly unlikely that the large number of resistant alleles needed for resistance to the high dose will initially be found in a single individual. In contrast, major gene resistance conferred by a single gene having a large effect rises exponentially at a rate that depends on the dosage (i.e. selection pressure) applied. Monogenically-resistant populations usually appear to burst forth suddenly after a number of successive exposures to high doses, as predicted in many models (Georghiou and Taylor, 1977a,b; Gressel and Segel, 1978). The weak selection pressure exerted by low doses increases the proportion of individuals in a population bearing major gene resistance at a much slower rate than does the strong selection pressure exerted by high doses.

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Continuing the old debate over major monogenic versus incremental quantitative inheritance as a primary cause of resistance is futile and moot as some organisms have evolved resistance by both mono- and quantitative genetic mechanisms (Crow, 1957; Galun and Khush, 1980; Putwain *et al.*, 1982; Lande, 1983; Raymond *et al.*, 1989; Devonshire and Field, 1991) and some organisms have evolved one or the other due to different pesticide or drug regimes (Putwain *et al.*, 1982; McKenzie *et al.*, 1992; Gressel, 1995b). This leads to a situation analogous to the double bind described in *Catch-22* (Heller, 1979); low doses that delay monogenic resistance select for quantitative resistance (Via, 1986; Shaw, 1989), while high doses that delay quantitative resistance bring about monogenic resistance. Major monogene resistance had been the prevalent cause of pesticide resistance until farmers started reducing doses (Gressel, 1995a; Gressel *et al.*, 1996).

1.1. *Selecting for/delaying major monogenic resistance*

Major monogenic resistance is often but not exclusively due to a modification in the target enzyme affected by the pesticide or drug. The target enzyme conformation is changed such that it can no longer interact with the pesticide or drug. Major monogenic resistance can also be due to a large change in the metabolic capacity to degrade or take up the toxin, as well as by yet unknown causes.

The early strategy of vast-overkill doses of antibiotics was quickly abandoned as it enhanced the selection of rare resistant individuals by removing all susceptibles (i.e. the antibiotics exerted extremely high selection pressure). Conversely, the use of very high rates of insecticides has been proposed for situations where resistance is semi-dominantly inherited on a major gene coding for a resistance, based on the type of dose responses seen in figure 1. A somewhat lower rate would not control heterozygotes, which can then inter-breed producing offspring, 25% of which will be homozygous and resistant to high dose-rates. This strategy is based on the concept that if the heterozygotes naturally occur at a low frequency (e.g. 10^{-6}) in a population, the homozygously-resistant individuals would theoretically be assumed to be found at a much lower frequency (e.g. $<10^{-12}$). The numbers of individuals required to test this assumption are huge. Many species are at too low a density to have resistant organisms within breeding distance of mates, defying the possibility to easily test the assumptions. In one effort using a lower eukaryote, the recessively inherited homozygous resistant mutants were found at a 10-fold lower frequency than the heterozygotes, i.e. 10^{-7} and not the expected 10^{-12} (Williams, 1976). Presumably some sort of somatic recombination mechanism allows for this contradiction of blindly accepted, but untested theory. Thus, it is not clear that there will be much benefit to using ultra high rates to control heterozygotes.

Lower doses were advised to lower selection pressure for monogenically inherited resistance, allowing the often more fit susceptibles to decrease the rate of evolution of resistance in populations (Gressel and Segel, 1978). Indeed, major gene resistance to triazines evolved first and mainly where high rates of the more persistent pesticides in this class were used, and rarely where low rates and/or less persistent triazine analogues were used.

When the enrichment of evolution of monogenically inherited resistance is plotted on a logarithmic scale (based on a simple population dynamics model), the increase is linear (figure 2). This is seen in the field as a sudden jump from a seemingly totally sensitive population to one where a high proportion of individuals is resistant to a high dose of the pesticide, as seen in figure 3(A). The farmer cannot tell when one pest in a million, or one in a hundred is resistant, only when a goodly proportion show resistance. The evolution of monogenically-inherited resistance follows the models for evolution of resistance previously proposed (Gressel and Segel, 1978; Maxwell *et al.*, 1990; Mortimer *et al.*, 1992). The pests are then resistant to massive doses of the pesticide that do not change with further treatments. This is because of the exponential enrichment of the resistant allele from its low natural frequency for a number of generations during which the increase in frequency of resistance is experimentally imperceptible (figure 2). As there is always a small proportion of susceptible individuals missed by the pesticide treatment, the resistant individuals are not noticed until they become about 30% of the whole population, and then cannot be ignored.

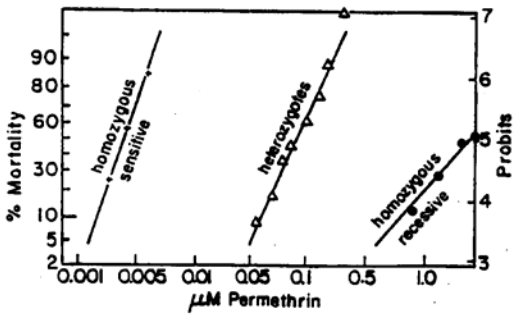

Figure 1. Dependence of resistance on dominance and dose. Dosage–response lines for larvae of Culex quinquefasciatus *homozygous susceptible, heterozygous, homozygous-recessive resistant tested with permethrin. Modified from Georghiou and Taylor (1986). Reprinted by permission of the American Chemical Society from Gressel (1995).*

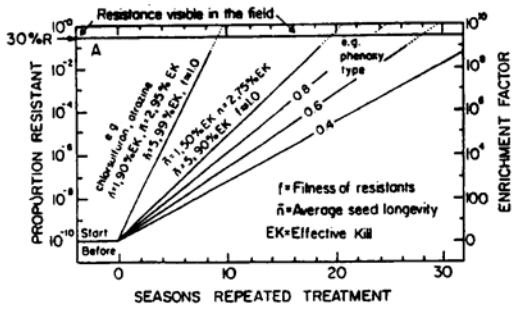

Figure 2. Evolution of major gene traits following repeated treatments of a pesticide. Modelling a scenario of successive treatments with highly persistent pesticides that controls throughout a cropping season (acute line) vs a short lived pesticide that allows the expression of the fitness difference between resistant and sensitive individuals after the pesticide has been dissipated (obtuse lines). Note logarithmic scale for increase. Based on equations in Gressel and Segel (1978).

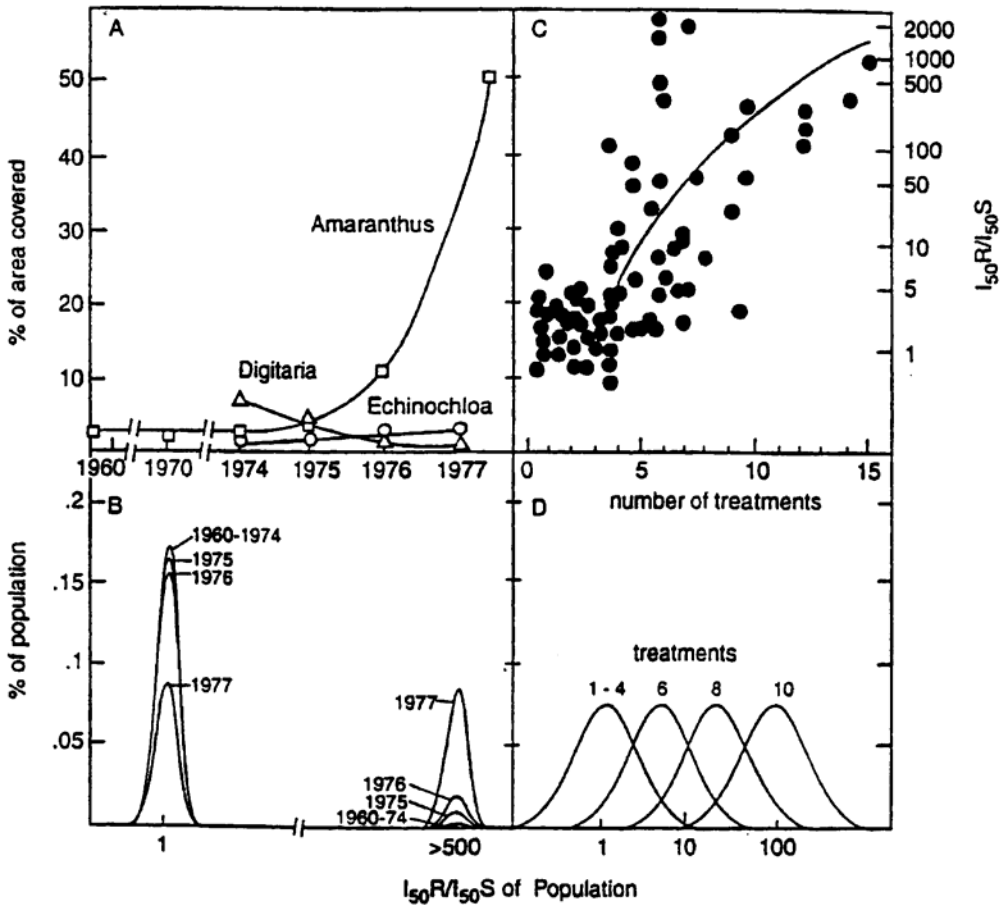

Figure 3. 'Sudden' appearance of major monogene resistance vs slow incremental creep of quantitatively inherited resistance. (A) Actual field data on resistance showing changes in weed populations in a monoculture maize treated annually with atrazine. Amaranthus retroflexus, Echinochloa crus-galli, *and* Digitaria sanguinalis, *the foremost weeds, were counted. The maize field was treated with atrazine from 1970 onwards (data are plotted from Table 1 in Nosticzius* et al. *(1979). (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 375 g/ha 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 Heap (1988). (D) A population distribution description of the data in (C) where the dose rates on the horizontal axis are arbitrarily plotted. Reprinted by permission of the American Chemical Society from Gressel* et al. *(1996).*

The strategy often suggested to delay target site resistance is to lower the dose rate (Gressel and Segel, 1978, 1982). This brings about a decrease in effective kill (EK in figure 2), which lowers 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 germinating susceptible members of the same weed species, or later influxes of the same species of fungi or insects, to dilute the proportion of resistant individuals in a population. 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 deal only with major gene effects (e.g. Comins, 1977; Gressel and Segel, 1978, 1982; Taylor, 1983; Roush and Daly, 1990) and only a few deal with polygenic resistance (e.g. Shaw, 1989) and gene amplification (Tabashnik, 1990). None have dealt with the simultaneous existence of both genetic mechanisms in the same organism.

1.2. Selecting for/delaying quantitatively inherited resistance

There are field data that justify the approach of not using low doses. Resistance quickly evolved when low dose-rates were used and quickly later evolved to stepped increases in dose

rates. Resistance has not yet evolved in parallel situations where high doses of the same pesticide were uniformly used. A recent cogent example can be seen in India, where the weed *Phalaris minor* evolved resistance to the phenylurea herbicide isoproturon in Green Revolution wheat growing areas. In the large 'pockets' (*ca* 30 km diameter) where this has occurred, farmers consistently underdosed the pesticide. Resistance has not yet evolved in nearby areas where full dose-rates were continually used. For example, in a typical documented case only half the recommended rate of isoproturon was initially used. This successfully controlled *Phalaris* for the first 3 years, but with inadequate control in the fourth. Then 0.75 the recommended rate was successfully used for 2 years, and unsuccessfully in the third. The full dose-rate was then successful for 1 year but inadequate the next. Fifty percent above the recommended rate worked for a year, but not after that. This strategy of continually increasing dose-rates might be feasible for some insecticides used in high value crops, but is less feasible for fungicides, herbicides and drugs where there is far less margin between a utilizable rate and toxicity to the crops or patients. For less valuable crops, economics can also play a role in limiting the rates used for any pesticide.

Where quantitative inheritance is involved, it has been shown time and again that the initial use of low dose-rates facilitates rapid evolution of resistance. The nature of quantitative inheritance is such that there are small increments of increase in resistance in such a population (figure 3(C)). Perhaps the appearance of a measurable proportion of individuals with the first increment of resistance is delayed (as in figure 2) until the first gene dose for resistance has been sufficiently enriched in the population. After the first increment of resistance appears, some individuals can withstand the evolutionary pressure of higher pesticide doses, enriching for more gene doses. Initial models on evolution of quantitative resistance to pesticides were described, but not fully developed (Via, 1986). It has recently been stated that 'the impact of quantitative trait locus studies on evolution has yet to be felt' (Cheverud *et al.*, 1996). Presumably they mean that while there is considerable circumstantial and epidemiological evidence for quantitative controls, the genetic proofs are minimal. In this respect they are clearly on target. For example, there are yet no direct genetic data to support that the resistance shown in figure 3(C) is quantitatively inherited, although there are data for quantitative inheritance in a similar case in the mild resistance to triazines in *Senecio vulgaris* (Holliday and Putwain, 1980), a species where monogenic target site resistance is also known. There is also some genetic evidence for quantitatively inherited incremental increases in some fungicide resistances (see Brent *et al.*, 1990; Holloman *et al.*, 1990; DeWaard, 1992), insecticides (see Mouches *et al.*, 1986; Field *et al.*, 1988), herbicides (Wang *et al.*, 1991) and anti-cancer drugs (see Schimke, 1984).

High initial doses have been proposed as an initial strategy to prevent quantitatively inherited resistance in cancer chemotherapy (Harnevo and Agur, 1991, 1992, 1993; Murray, 1995), because low and then increasing doses have been shown to select for amplification (Schimke, 1984). In the case of anti-cancer drugs, this modelling has suggested that after the first high initial doses are used, the dose can actually be dropped due to an interplay between 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 compete successfully with late germinating weeds. This strategy has the potential problem in medicine of high doses being lethal to a certain portion of patients, and in agriculture of cost and phytotoxicity to the crop.

1.3. Conceptual underpinnings of the revolving dose model

In this paper, we model a situation where resistance may evolve by either of the two genetic mechanisms. In major monogenic resistance there is a single major locus with two alleles *R* and *r*, and *R* confers resistance. The second source of resistance is quantitative, where each minor gene, gene amplification, or series of mutations in a single gene each contributes a small increment of resistance. The total resistance found in an individual is the sum of the contributions of all of these incremental changes. In many cases, each minor gene contributes to the metabolic degradation of the pesticide, its exclusion or its being sequestered, or a mixture of such mechanisms. The more gene doses, the higher dose of the chemical the organism can withstand.

Quantitative resistance can be more problematic than monogenic resistance because organisms with quantitative resistance often have cross-resistances to totally unrelated chemicals. The quantitative component of the model follows the standard assumption of quantitative genetics that the resistance level in a population will follow a Gaussian distribution with fixed variance in every generation (Falconer, 1989), i.e. the median effective dose of pesticide or drug will incrementally and continuously creep up to higher levels, as seen in figure 3(D).

We propose a strategy to partially overcome the Catch 22 situation: applying a series of low doses punctuated at regular intervals with an intermediate dose, in a rotating pattern. This strategy delays both types of resistance longer than continuously applying either a series of only low doses or a series of only high doses. The key notion is that when quantitative resistance begins to build up after the series of low doses, the intermediate dose eliminates individuals with quantitative resistance before they accumulate enough of the small genetic changes to resist that intermediate dose. This resets quantitative resistance back to near its original low level, as the only survivors are the rare individuals with monogenic resistance selected at this intermediate dose with a moderate selection pressure.

The size of the pest population, however, affects the probability that there will be individuals with sufficiently high quantitative resistance to survive the intermediate dosage, so even low doses must be adequately high to keep pest populations in check. The model suggests that monogenic resistance should emerge more slowly with rotating doses than if exclusively intermediate or high doses were applied. We assume that the pest population growth rate is density dependent. The rotation strategy was described empirically (Gressel, 1995b; Gressel *et al.*, 1996), and we now present the mathematical underpinnings of this concept.

In cases where alternative control measures are possible, such as rotating crops or pesticides, we advocate such rotations as better all around management strategies. However, some crops cannot be easily rotated (such as wheat due to extreme

climate or soil conditions, or orchards) and no alternative pesticides exist. As Denholm and Rowland (1992) summarize: 'Resistance is now frequently multifactorial, involving a suite of coexisting mechanisms with contrasting cross-resistance patterns that protect organisms against the same or different classes of pesticides and may even predispose them to resist new, as yet unused compounds' (p. 92). Then pest control might well consist of carefully planned dosage strategies.

1.4. Lolium, *the example for the model*

We developed and based the revolving dose model using the example of ryegrass (*Lolium*), a common weed in wheat fields. Ryegrass is a good model species, as both quantitative and major gene resistance have plagued wheat farmers around the world, no practical alternative pesticides are presently available, and wheat is often the only cash crop capable of growing in regions inhospitable to other crops, leaving farmers and consumers in the lurch when resistance appears.

In the US and Canada, farmers sprayed the herbicide diclofop-methyl at the high rate of 1200 g/hectare. Resistance arose at many geographic locales, and researchers traced the monogenic source to be a modified acetyl-CoA-carboxylase, the target enzyme of the herbicide (Gronwald *et al.*, 1992). Inheritance of monogenic resistance is semi-dominant, but functionally dominant at the rates of pesticide that can be used in the field. Higher rates of pesticide application would require homozygosity for resistance and therefore prevent the build-up of the resistant allele in heterozygotes. Higher rates, however, would also kill the wheat, as well as being economically prohibitive.

In Australia, economics precluded this high dose, so farmers sprayed the lower dose of 375 g/ha of the same herbicide. This rate sufficiently, although incompletely, controlled previously untreated populations of ryegrass. In the last decade, thousands of scattered cases of resistance were found, now covering more than a third of Australian wheatlands. The Australian populations initially did not have major monogenic resistance, and different populations had different qualitative and quantitative cross-resistances to other herbicides with different chemistries (Heap, 1988; Preston *et al.*, 1996). The level of resistance within populations slowly increased as a function of the number of treatments as an upward creeping mean (figure 3(C and D)), which is typical of quantitatively inherited traits.

Early on it was posited that the resistance in Australia was due to elevated levels of enzymes capable of degrading herbicides, probably one or many NADPH dependent cytochrome P450 monooxygenases (cyt P450s), similar to those present in wheat (Gressel, 1988). Wheat is known only to use monooxygenases to naturally degrade the selective herbicides used in this crop. Indeed, indirect evidence has shown that the Australian *Lolium* uses cyt P450s as well as other possible mechanisms (Preston *et al.*, 1996). The structural genes for cyt P450s are known to exist in large gene families containing tens to hundreds of genes (Bolwell *et al.*, 1994). They have been described in insects (Bradfield *et al.*, 1991; Gandhi *et al.*, 1992; Feyereisen *et al.*, 1995) and plants (O'Keefe *et al.*, 1991) although they are best described in mammalian systems. Single gene mutations in the structure of cyt P450 are known to modify substrate specificities. Additionally, genes that control the expression levels (synthesis and turnover) of groups of cyt P450s are known. Other pesticide detoxification mechanisms such as glutathione transferases can also be under quantitative controls. The vast arrays of different cross resistances to other herbicides in *Lolium* (Heap, 1988; Powles, 1993; Preston *et al.*, 1996) support quantitative inheritance. Despite, or perhaps because of, the wide array of cross-resistance spectra, we cannot presently estimate how many of the possibly hundreds of available genes contribute to any given case of resistance. Similar large differences in fungicide cross-resistances have been shown to be controlled, in some cases, by many independent genes (Peever and Milgroom, 1993).

Most models for resistance have been influenced by laboratory generated dose–response curves using laboratory cultivated pest populations. We now realize that this can give a somewhat erroneous picture of the field or hospital situation. A pesticide or drug 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. Additionally, in the laboratory pests at highly uniform age are treated uniformly, giving equal distribution of pesticide. In the laboratory 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 (figure 4). 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 laboratory, and various instars are treated simultaneously. Again a skewed dose–response probit curve will be obtained.

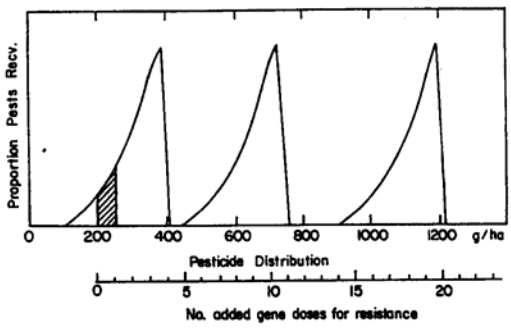

Figure 4. Presumed distribution of a pesticide on pests following spraying in the field at 400, 750, 1200 g/ha, illustrating the proportion of pests receiving each dose. Double spraying is ignored, as are untouched escaped organisms (in 'refuge'). An added scale shows how many additive independent mutant gene dosages would be required to withstand each dose. Assumptions: each mutant gene dose provides protection for 50 g/ha beyond the threshold of 200 g/ha. The cross-hatched area shows the sensitive population from which one gene dose will be selected. Reprinted by permission of the American Chemical Society from Gressel (1995).

Fungi at different stages of development, germination, penetration and establishment are differently affected by fungicides. Different age or size tumours have different drug susceptibilities and penetration. These variabilities would all 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 figure 4. 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 375 g/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 200–250 g/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 figure 3(C) 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 successive treatments of pristine populations with diclofop-methyl at low rates in Australia, there were often *Lolium* plants that appeared very sick or even 'dead'. Many such sick plants later recovered to produce some seed (Ian Heap, personal communication). These may well be the plants with the first resistant polygenes 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 1200 g/ha is used, it is clear that >97% of the pests are killed (figure 4). Most of the survivors were in refuges and received no pesticide at all. An infinitely small proportion of plants received 250–300 g, so that the selection for a single resistant polygene would be minimal. Assuming one polygene is required on average for every 50 g of pesticide above 200 g/ha, then 20 polygenes would be needed to survive 1200 g/ha. There would theoretically be one plant with 20 polygenes at a frequency of $10^{-4\times20}=10^{-80}$ 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 (Falconer, 1989). If a pristine population has a normal distribution of polygenic resistance centred 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 code on a major gene. If the field is treated with a 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.

1.5. *From example to model*

Field experiments to find optimal pesticide dosage strategies for resistance management of each new pesticide or drug would be expensive, require impractically long durations and many individual organisms, and produce a distressing number of populations of resistant pests. In contrast, modelling enables us to predict whether it is possible to delay the evolution of resistance for long periods, what levels and rotations of low and high pesticide doses will do so, and which of these combinations will minimize the total pesticide usage over a period of treatments. Such models could be tested with a lower number of treatments than direct empirical testing.

We consider the effects of three factors on the success of dosage rotations: the mode of selection, the dominance of the major gene for resistance, and the heritability of quantitative resistance. First, we compute results for a population subjected to truncation selection. This is a situation where selection acts as a knife-edge on survivorship, so that all individuals with resistance greater than the dosages applied survive, and those with resistance lower than the dose applied die (figure 5). This situation fits insects that attack cotton in Australia where all growers use the same insecticides, and the insect pest has no secondary hosts (Forrester and Bird, 1996). It also applies to flightless scale insects infesting orchards and many grass weeds, including the ryegrass *Lolium* in minimal tillage agriculture where weed seeds do not become incorporated into the soil and germination is in a single flush. The truncation selection case requires that the pest population has a uniform dose–mortality relationship, which usually occurs in the laboratory when the pests are grown synchronously, i.e. insects in the same stadium or weed seedlings having the same number of leaves.

Instead of truncation selection, a population may be subject to smooth selection (figure 5). This situation is similar to the first, except that it recognizes that pesticides in the field cannot be applied as evenly as in the laboratory, so that not all pests receive a uniform dosage. Those individuals that are sheltered from pesticide treatment by leaves or clods of dirt do not experience such strong selection for resistance. Moreover, pests of different ages have different levels of natural resistance to given pesticides (Carey, 1982; Klingman and Ashton, 1982). In addition, pesticides often persist for long periods at declining levels. Thus, some individuals emerging late encounter lower residual doses than the applied dose and may survive despite lower resistance. Our analyses based on smooth selection therefore apply to situations where pesticide application is very uneven, pests vary in their susceptibility due to developmental

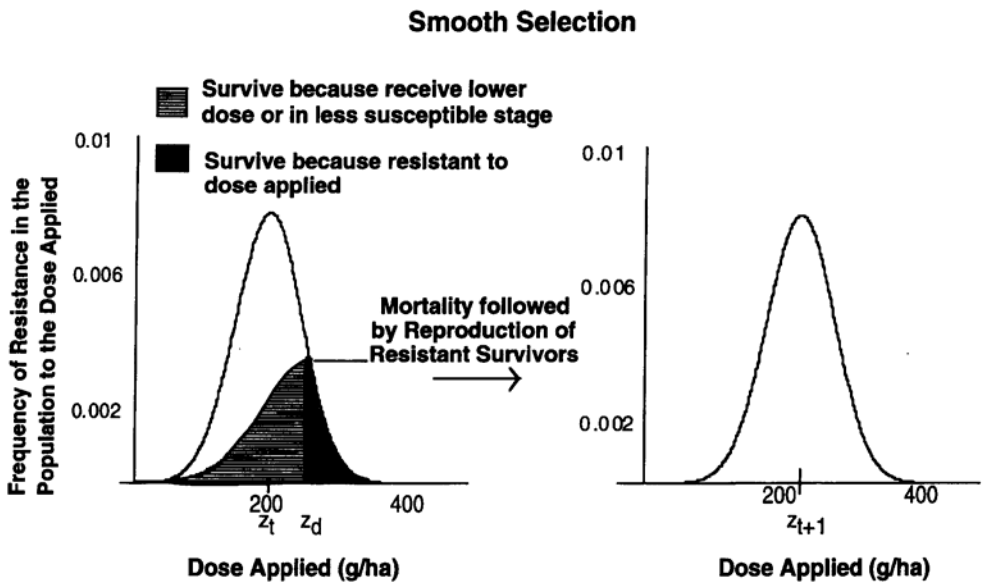

Figure 5. The level of quantitative resistance of the population shifts under selection followed by reproduction of the survivors. Under truncation selection, the only survivors are those with resistance to the dose applied, shown in the shaded region. Under smooth selection, in addition to the small proportion of survivors that are truly resistant to the full dosage applied, some individuals survive because they receive an insufficient amount of pesticide (e.g. because of uneven application in the field) or are at a less susceptible stage of development, shown in the horizontally striped region. The distribution of resistance of the population has mean z_t before selection with dosage of pesticide z_d that is toxic to a proportion of the population, and the distribution shifts to mean z_t+1 after selection and reproduction by the survivors. The values on the x-axis refer to an arbitrary low dose akin to the Lolium example.

or behavioural differences, and/or the residual toxicity of the pesticide declines.

We compare results if the major gene for resistance is completely dominant, partially dominant, or recessive, using the standard equations for selection at a single locus with two alleles (Crow, 1986). Changes in gene frequency are computed based on the relative fitnesses of the homozygotes and the heterozygote, where fitness is given by the dose–response curves for each genotype.

Finally, we incorporate alternative assumptions about the impact of selection on heritability. One possibility, is that heritability changes over time due to linkage disequilibrium generated from truncation selection (Bulmer, 1980), but that heritability does not vary as a result of altered allele frequencies, since such changes are unpredictable. Linkage disequilibrium, the inheritance of groups of genes linked on the same chromosome, results in an initial decline in heritability after truncation selection. Recombination, which breaks apart non-random associations among linked genes during subsequent generations of mating, causes heritability to gradually rise back to an equilibrium level. We also modelled another possibility, that heritability is low initially in a pristine population, then rises with selection as rare resistant alleles or combinations of genes become more frequent, then declines again as susceptible alleles or combinations of genes become more rare under continued selection.

In future analyses, we will consider situations in which a substantial number of susceptible immigrants from untreated populations enter the selected population in each generation, or in which individuals may escape pesticide treatment by remaining dormant in a refuge (e.g. a seedbank).

2. Methods

2.1. The model

In the model, we assume a monoculture system where the same pesticide is used each season/generation of pests. After application of the pesticide, the resistant survivors mate, producing offspring with a Gaussian distribution of quantitative resistance whose mean is shifted to a level that depends on the dosage of pesticide applied. Selection for major gene resistance increases the frequency of *R* among the offspring at a rate determined by the relative fitnesses of the genotypes *RR*, *Rr* and *rr*. We assume random mating. The notation used in the description of the model is summarized in table 1.

2.2. Major gene resistance

The enrichment of resistant allele *R* that codes for a major change in major gene resistance follows the dynamics of single-locus, two-allele genetics. The relative fitnesses of the resistant homozygote (W_{RR}), heterozygote (W_{Rr}), and susceptible homozygote (W_{rr}) are given by 1, 1-ks(z_d), and 1-s(z_d), respectively, where $s(z_d)$ is the dose dependent strength of selection against susceptible individuals and k is the degree of dominance of *R* over *r*. When $k=0$, the resistance gene is dominant, when $k=0.5$ then *R* is semi-dominant and the allelic affects are

Table 1. Explanation of notations used

Parameter	Definition
z_d	Dosage applied
p_t	Frequency of the resistant allele for major gene resistance in generation t
q_t	Frequency of the susceptible allele for major gene resistance in generation t
$s(z_d)$	Strength of selection from a dose z_d against susceptible homozygotes (rr) relative to resistant homozygotes (RR)
k	Degree of recessiveness of major allele for resistance (k=0 is dominant, k=0.5 is intermediate or additive, and k=1 is recessive)
$p(z,z_d)$	Fraction of individuals with quantitative resistance z that survive a dose z_d
z_t, σ_t	Mean and standard deviation of the distribution of quantitative resistance of the population in generation t before selection
z_{tw}	Mean quantitative resistance after selection in generation t but before reproduction
$h^2(t)$	Heritability of quantitative resistance in generation t
$V_A(t)$	Additive genetic variance after t generations of selection
V_E	Environmental variance
h^2_{max}	Maximum value at which heritability peaks, in the case where $h^2(t)$ depends on z_t
σ_s	Parameter describing how gradual the selection curve is in the case of smooth selection
$w_q(z_d)$	Fraction of the population that survives a dose z_d as a result of quantitative resistance
$w_m(z_d)$	Fraction of the population that survives a dose z_d as a result of major gene resistance
$w_{tot}(z_d)$	Total fraction of the population that survives a dose z_d as a result of either quantitative or major gene resistance
$N(t)$	Size of the pest population in generation t
λ	Growth rate of the pest population

additive, and when $k=1$ then R is recessive. If p_t is the frequency of the R gene in generation t, and q_t the frequency of r, the change in p after one generation of selection is given by (Crow, 1986):

$$p_{t+1} = \frac{p_t^2 + p_t q_t(1 - ks(z_d))}{w_m(z_d)} \quad (1)$$

where the mean fitness $w_m(z_d)$ is

$$w_m(z_d) = p_t^2 + 2p_t q_t(1 - ks(z_d)) + q_t(1 - s(z_d)) \quad (2)$$

In our formulation, $w_m(z_d)$ is equivalent to the fraction of the population that survives a dose z_d because those individuals either contain a resistant allele or because $s(z_d) < 1$ (the dose is low enough that some susceptibles survive).

The initial monogene frequency p_0 results from the natural frequency of mutation of the gene, taking into account its steady state loss due to relative unfitness or random drift in an untreated population. In most of the computations that follow (unless specified otherwise), we assume that $p_0 = 10^{-6}$ and that:

$$s(z_d) = \log(1 + 0{\cdot}0015z_d)_+ \quad (3a)$$

where the '$_+$' indicates that s is truncated at 1 (figure 6). We also examined results obtained using the alternative curve

$$s(z_d) = 1 - e^{-0{\cdot}003z_d} \quad (3b)$$

The factors 0.0015 in equation (3a) and 0.003 in equation (3b) scale the dose to relevant units, but do not affect the shape of the selection curve, so can be altered without changing the results. In both cases (equations (3a) and (3b)) selection against susceptibles is monotonically increasing with dose and concave. We wished to compare two dose–response curves with this same general form but with slightly different shapes to verify that the model predictions are not overly dependent on the specific form of the selection curve, since this may be difficult to measure in the field. We also compared rotations versus constant doses when $s(z_d)$ was convex, increasing as the square of dose

$$s(z_d) = (0{\cdot}001z_d)^2 \quad (3c)$$

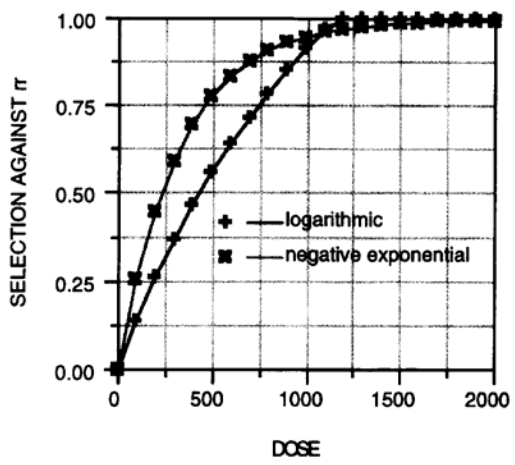

Figure 6. Selection $s(z_d)$ against homozygote susceptibles (genotype rr) versus the dose z_d applied. Two alternative functional forms are plotted, logarithmic (equation (3a)) and negative exponential (equation (3b)).

In addition, we examined the robustness of model results to variation in the initial frequency of the resistant allele (p_0) and the heterozygous effect (k).

2.3. Quantitative resistance

We assume that a large number of possibly mutated genes each contributes a small amount of resistance. The total level of resistance is the sum of this small amount over the large number of genes. With only 20 genes, any quantitative system behaves approximately as if there were an infinite number of genes (Thoday and Thompson, 1976). We assume that in generation t, the fraction $f_t(z)$ of individuals with resistance z is normally distributed with mean $\bar{z}_t$ and phenotypic variance σ_t^2

$$f_t(z) = \frac{1}{\sigma_t\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{z-\bar{z}_t}{\sigma_t}\right)^2} \quad (4)$$

Initially, $\bar{z}_0 = 200$ g/ha. *Phenotypic variance* (σ_t^2) is the sum of the additive genetic variance ($V_A(t)$) and environmental variance (V_E). We assume that there is no non-additive genetic variance and that $V_E = 80$ is constant over time, but that $V_A(t)$ changes with selection from linkage disequilibrium or altered allele frequencies (see below). Then $\sigma_t^2 = V_A(t) + V_E$. Narrow sense heritability is $h_t^2 = V_A(t)/\sigma_t^2$.

If $p(z,z_d)$ is the fraction of individuals with resistance z surviving a specified dose z_d due to quantitative resistance, then the total fraction of the population surviving a dose z_d due to quantitative resistance is

$$w_q(z_d) = \int_{-\infty}^{\infty} p(z, z_d) f_t(z) dz \quad (5)$$

2.4. Type of selection on quantitative resistance: truncated or smooth

For the case in which survival is truncated, so that no individuals with resistance beneath the applied dose survive, the function describing the fitness of individuals with resistance z to a dose z_d is

$$p(z, z_d) = \begin{cases} 0 & z < z_d \\ 1 & z \geq z_d \end{cases} \quad (6)$$

Substituting $p(z,z_d)$ into equation (5) yields survival under truncation selection, denoted by $w_{q,trunc}(z_d)$, which can be rapidly evaluated using the algebraic approximation (Abramowitz and Stegun, 1965) for the cumulative Gaussian distribution.

For the situation in which selection is smooth, allowing a portion of individuals with resistance below z_d to survive, the probability of survival gradually increases with the level of resistance. All individuals with resistance greater than, or equal to, z_d survive, so that

$$p(z, z_d) = \begin{cases} \exp\left(-\frac{(z-z_d)^2}{2\sigma_s^2}\right) & z < z_d \\ 1 & z \geq z_d \end{cases} \quad (7)$$

In contrast to truncation selection, under smooth selection some individuals survive because they receive less than the dosage applied or, because of asynchrony in growth, they are in a less susceptible stage of development. This can allow for selection and enrichment of polygenes conferring low levels of resistance but sufficient to combine and later confer resistance to higher dose levels. The parameter σ_s determines the smoothness, or width, of the selection curve. In the limit as σ_s goes to 0, we return to the case of truncation selection. In the limit as σ_s goes to infinity, there is no selection since $p(z,z_d) \to 1$ for all z values. Unless specified otherwise, $\sigma_s = 50$. We denote the mean survival under smooth selection for individuals with resistance $z < z_d$ by $w_{q,smooth}(z_d|z<z_d)$. The mean survival of individuals with $z \geqslant z_d$ is the same as under truncation selection, $w_{q,trunc}(z_d)$. Hence, the total survival over all z values for smooth selection is $w_{q,smooth}(z_d) = w_{q,trunc}(z_d) + w_{q,smooth}(z_d|z<z_d)$. The equation for calculating $w_{q,smooth}(z_d|z<z_d)$ is derived in Appendix A.

2.5. Dynamics of the quantitative mean resistance

The mean quantitative resistance of the population after selection but before reproduction is

$$\bar{z}_{tw} = \frac{\int_{-\infty}^{\infty} z p(z, z_d) f_t(z) dz}{w_q(z_d)} \quad (8)$$

We derive equations for the calculation of $\bar{z}_{tw}$ under truncation selection and smooth selection (see below) in Appendices A (smooth selection) and B (truncation selection).

To compute the dynamics of the mean following reproduction, we use the fundamental equation of quantitative genetics. However, we modify this equation slightly to account for the fact that the population is finite, so that if survival is very low then no individuals with quantitative resistance are likely to survive. The size of the population in generation t is $N(t)$. If at least one individual with quantitative resistance survives ($w_q(z_d) \geqslant 1/N(t)$), then the usual equation for the mean holds:

$$\bar{z}_{t+1} = \bar{z}_t + h^2(\bar{z}_{tw} - \bar{z}_t) \quad (9)$$

In this equation, $\bar{z}_{tw}$ is the mean after selection but before reproduction and $\bar{z}_{t+1}$ is the mean after selection and reproduction. Although theoretically, some proportion of the population lives even if the surviving fraction is smaller than $1/N(t)$, in reality individuals are discrete rather than continuous entities. It is improbable that any will live if the chance of survival is too low since the population is finite. Thus, when it is unlikely that a single individual with quantitative resistance survives the pesticide application ($w_q(z_d) < 1/N(t)$), the mean is reset to a low starting value

$$\bar{z}_{t+1} = \bar{z}_0 \quad (10)$$

The interpretation of equations (7) and (8) is that if there is a sufficient number of survivors then the mean follows the usual dynamics of quantitative genetics. In this model, immigration of susceptible pests is negligible, so any immigrants contribute nothing to the mean resistance as long as some resistant residents survive to outcompete the immigrants in the presence of the pesticide. However, if no residents survive, then for the pest population to persist it must be replenished by untreated immigrants.

We assumed the most conservative case (requiring the highest dose to reset the mean quantitative resistance), in which only one individual needs to survive to preserve alleles for resistance. This might be the case for a self-fertile weed. If more than one individual needs to survive, as for an outbreeding pest, then the mean may be reset despite a higher proportion of survivors $w_q(z_d)$, and so a lower dose will suffice.

2.6. Changes in heritability with recurrent selection

We consider two cases for the change in heritability with each episode of selection: change due to linkage disequilibrium, or change due to the accumulation of alleles conferring resistance. In the first case, selection alters additive genetic

variance due to departures from linkage equilibrium (Bulmer, 1980). While linkage disequilibrium is not responsible for changes in the mean in the absence of epistatic interactions (such changes result instead from altered allele frequencies), linkage disequilibrium may influence the response to selection by changing heritability.

Heritability after t generations of selection is

$$h^2(t) = \frac{V_A(t)}{\sigma_t} \tag{11}$$

When $w_{q,trunc}(z_d) \geqslant 1/N(t)$ the additive genetic variance after $t+1$ generations of selection is (Bulmer, 1980)

$$V_A(t+1) = \frac{1}{2}[1 - h^2(t)c(c-y)]V_A(t) + \frac{1}{2}V_A(0) \tag{12}$$

where

$$y = \frac{z_d - \bar{z}_t}{\sigma_t} \quad \text{and} \quad c = \frac{\frac{1}{\sqrt{2\pi}} e^{-\frac{y^2}{2}}}{\bar{w}_q(z_d)} \tag{13}$$

(see Bulmer, 1980, for derivation). When $w_{q,trunc}(z_d) < 1/N(t)$ then $V_A(t+1) = V_A(0)$.

Instead, heritability could depend on the mean resistance $\overline{z_t}$. When resistance alleles are at low frequencies, additive genetic variance, and therefore heritability, is also low. Initially, heritability rises with the frequencies of resistant alleles, but then declines again as alleles near fixation. We modelled this as the downward-facing parabola

$$h^2(t) = h^2_{max}\frac{(a - \bar{z}_t)\bar{z}_t}{(a/2)^2_+} \tag{14}$$

with a peak at $a/2$, where '$_+$' indicates that $h^2(t)$ is truncated at zero. Unless stated otherwise, for most of our analyses we assume heritability follows equation (14) with $a = 1200$ and $h^2_{max} = 0.5$, so heritability reaches a maximum value of 0.5, which occurs at the point where the mean resistance of the population is three times that of a pristine population (600 g/ha). We examine how different values of the peak heritability of quantitative resistances affects model predictions.

2.7. Total survival

The total proportion of the population surviving from major gene and quantitative resistance is the sum of survival from the two, correcting for the chance that an individual possesses the genes that confer both types of resistance (assuming the two are independent)

$$w_{tot}(z_d) = w_m(z_d) + w_q(z_d) - w_m(z_d)w_q(z_d) \tag{15}$$

2.8. Size of the pest population

The population size $N(t)$ in generation t is density dependent, following a discrete analogue of logarithmic growth

$$N(t+1) = \{N(t) + N(t)\lambda[1 - N(t)/N_{max}]\}w_{tot}(z_d) \tag{16}$$

where $N_{max} = 10^6$ is the maximum size (carrying capacity) of the pest population. If $N(t) = N_{max}$ then population size the following generation depends only on survival $w_{tot}(z_d)$ of drug dose z_d. If $N(t) < N_{max}$ then the population in $N(t+1)$ depends on both the rate of increase $\lambda(1\text{-}N(t)/N_{max})$ and $w_{tot}(z_d)$. Unless specified otherwise, $\lambda = 1$.

2.9. Computations

The modelling of the theory just described is easily performed on a microcomputer and can be adapted with different parameters in other pest and drug management strategies.† The rotation pattern is the frequency that the intermediate dose z_{medium} is applied in treatments between the small dose z_{small}. For example, if the rotation r is three, z_{small} is applied for two treatments followed by z_{medium} in the third, followed by another two treatments with z_{small} and one with z_{medium}, etc. We assume a population to be effectively resistant when either the quantitative mean resistance surpasses 600 g/ha or the frequency p_t of the R allele reaches 0.30. This fraction is approximately the level at which resistance becomes blatantly evident in the field (Gressel and Segel, 1978).

3. Results

One has to apply enough pesticide to keep pest numbers low, yet not so much that the pests rapidly evolve resistance. We plotted the timecourse of resistance and population size for two alternative tactics of using the same moderate dose of 700 g/ha in every treatment, or rotating low (300 g/ha) and high (1000 g/ha) doses every other treatment (rotation = 2; figure 7). Using a constant dose of 700 g/ha causes the allele conferring major gene resistance to become prevalent after 10–12 treatments if it is dominant and after slightly longer if it is only partially dominant, but the increase is undetectable if it is recessive (figure 7(A)). The population rapidly evolves resistance through quantitative mechanisms (figure 7(B)), causing the initially poor survival rate to rise quickly (figure 7(C)). The size of the pest population is suppressed by the pesticide for a short time, about eight treatments, and then explodes to the carrying capacity of the habitat (figure 7(D)). Whether the major allele is dominant or recessive makes little difference in the number of treatments before the population reaches its maximum size.

When alternating dosages of 300 and 1000 g/ha are applied, major gene resistance evolves after about the same amount of time as using the constant 700 g/ha (figure 7(E)). However, quantitative resistance never builds up to the same high levels as with a constant dose (figure 7(F)). Less than 30% of the population survives each low dose, and then partially resistant individuals are killed by the high dose before their offspring have an opportunity to gradually acquire full resistance to such a high dose. This resets the quantitative mean level of resistance. In treatments with the low dose, the major gene resistance rises more slowly than in high dose treatments, thus retarding its rise above 30%. When R is dominant, then for the first 12 treatments survival of the low dose is dictated by individuals with quantitative resistance, and thereafter by those with major gene resistance (figure 7(G)). Survival of the high dose is always determined by the frequency of the major allele. When Rr heterozygotes have fitness intermediate between RR and rr

†Email s.n.gardner@ic.ac.uk for such a program in True Basic.

homozygotes, major gene resistance takes longer to evolve, although the qualitative pattern of resistance evolution and population control is similar to the case when *R* is dominant. This is true even if *Rr* is nearly, but not quite, recessive ($k = 0.9$).

Comparing figures 7(C) and 7(G) illustrates the model prediction that dosage rotations can depress pest survival to a lower level and for twice as long as using a constant dose if *R* is dominant, and for much longer than constant doses if *R* is recessive. Delayed evolution of resistance results in successful pest control for more treatments using dosage rotations than using constant doses (figure 7(H)). This is the case whether or not *R* is dominant. In fact, the biggest advantage of rotation is seen when *R* is recessive, since selection for major gene resistance cannot occur on heterozygotes, and homozygous *RR* individuals are initially so rare that major gene resistance takes very long to evolve. Therefore, very high doses can be used to reset the

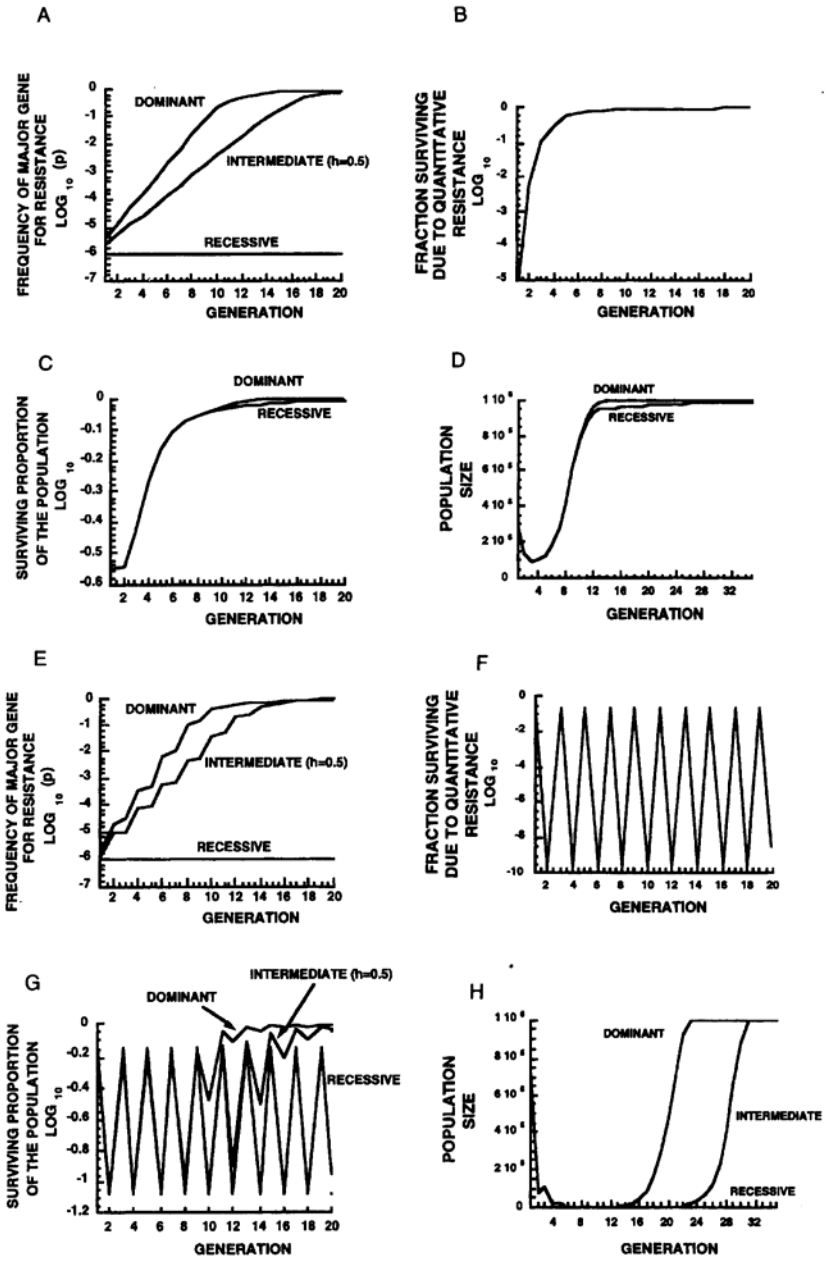

Figure 7. The rise versus pest generation number of (A) and (E) the frequency of the major allele R *for resistance when* R *is dominant, intermediate, or recessive; (B) and (F) the fraction of the population surviving as a result of quantitative resistance; (C) and (G) the total fraction of the population surviving due to either quantitative or major gene resistance, for dominant, intermediate, or recessive* R*; and (D) and (H) the size of the pest population. Parts (A) – (D) are when a dose of 700 g/ha is applied to every pest generation, and parts (E) – (H) are when a dose of 300 g/ha and 1000 g/ha are applied every other generation.*

quantitative mean resistance to a low level without the danger that major gene resistance will evolve quickly as a consequence.

For dose rotations to succeed, the high doses must be applied sufficiently frequently and be adequately large (figure 8). If high doses are applied too infrequently, quantitative resistance surpasses 30% before the high dose is applied, and then the high doses are no longer capable of fully resetting the quantitative mean (figure 8(A)). Pest populations become enormous during the series of low doses (figure 8(B)). However, there is a fair amount of leeway in the frequency at which high doses are applied; in figure 8(A and B) the rotation is a high dose every five treatments, although for more frequent high doses (rotation of 2–4) the rotating schedule is capable of resetting quantitative resistance. Similarly, high doses which are not sufficiently high cannot reset the quantitative mean (figure 8(C)), resulting in an earlier rise in pest numbers than would occur if the high dose had been greater (figure 8(D)). The risk of using an insufficient high dose is greater than the risk of an

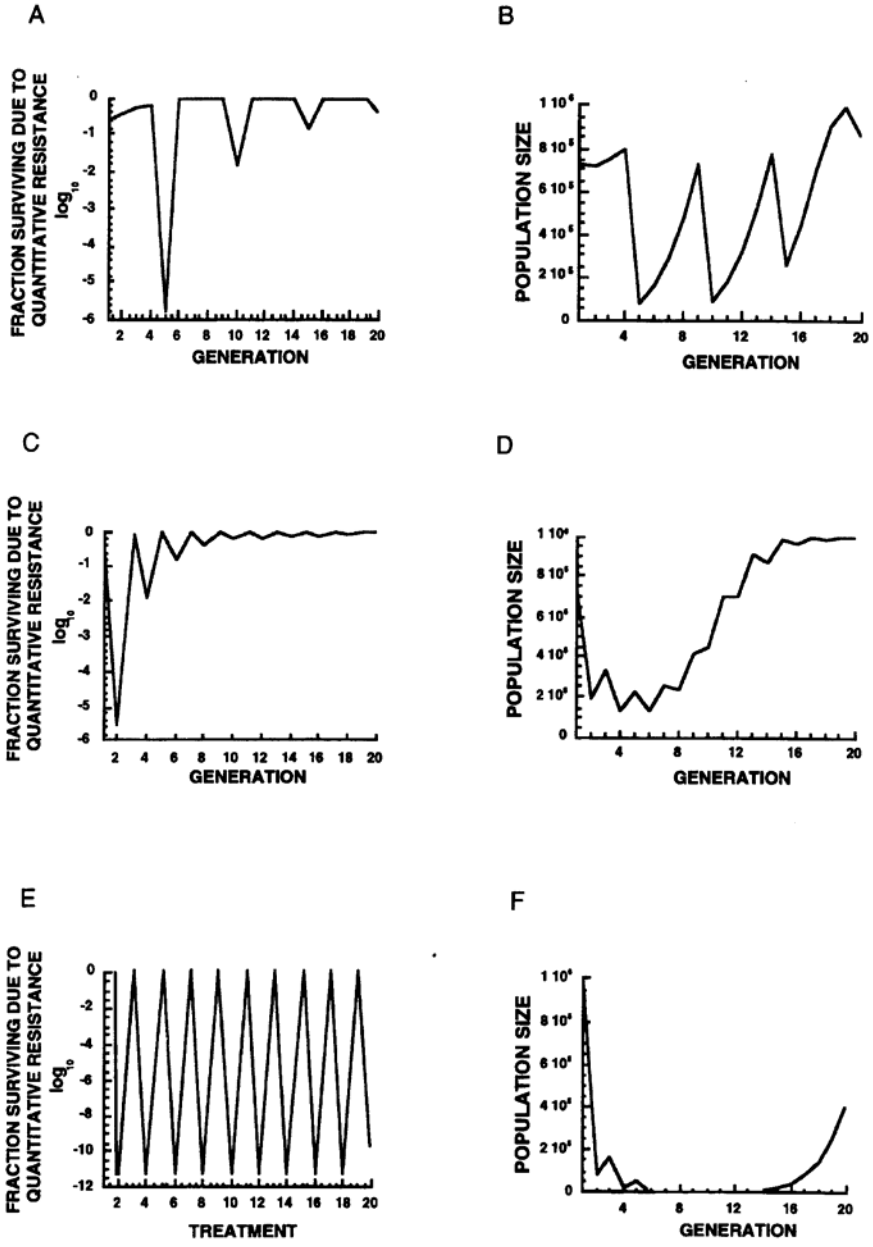

Figure 8. Effects of (A) and (B) high doses that are too infrequent, with a dose of 1000 g/ha applied in every fifth generation, and 300 g/ha applied in the other generations (i.e. rotation of 5); or (C) and (D) high doses that are too low, with a high dose of 800 g/ha applied every other generation, alternated with 300 g/ha, and (E) and (F) reducing the low doses to 100 g/ha, alternated with a high dose of 1000 g/ha. In (A), (C), and (E) the fraction of the population surviving as a result of quantitative resistance versus pest generation number; and (B), (D), and (F) population size versus pest generation.

inadequate low dose. If the low dose is too low, then, although many pests survive during the low years (figure 8(E)), the population never has a chance to recover from the intermittent high doses so remains quite small until major gene resistance evolves (figure 8(F)).

Figures 7(D and H) and 8 illustrate that a pest population may be controlled for a short period of time (less than 10 treatments) by both constant and rotating doses, but that doses must be selected more carefully to extend the success of population control for as long as 20 treatments. To summarize results from many dosage combinations, and to identify the dosage combinations that result in the smallest pest populations after prolonged pesticide applications, we created contour plots of population size (z-axis coming out of the page) after 15 or 20 treatments versus first (x-axis) and second (y-axis) doses, with a rotation of two (figure 9). Ideally, one would like to minimize the pest population size after 20 treatments, and use the least total amount of pesticide to do so. The total amount of pesticide applied over one rotation is the first dose plus the second dose, and pesticide isoclines are diagonal lines with slopes of -1 (heavy dashed line). The light dashed line with slope 1 describes a non-rotating dosage strategy. The mor e extreme the rotating strategy, the farther are points from this line.

In figure 9(A), illustrating (the very similar) results from both the case of recessive R after 20 treatments as well as the case of dominant R after 15 treatments, a pest population is predicted to be at carrying capacity, unhindered by pesticide, for first and second doses of less than 700 g/ha. For slightly higher doses, population suppression improves by an order of magnitude. However, using rotating doses enables one to use less pesticide to control the pest population. For example, one could apply 1000 g/ha every other treatment (1000, 0) and achieve the same pest suppression, with 800 g/ha per rotation less of pesticide, as using 900 g/ha twice as often (900, 900). The concave contours indicate that a rotating strategy can result in a smaller pest population than do constant doses.

After more treatments, population control begins to break down if the allele R confers some resistance in heterozygotes ($k<1$; figure 9(B)), as major gene resistance becomes more common. Nevertheless, the model predicts that pest populations can still be suppressed with less total pesticide by using dosage rotations. Moreover, if in the low-dose generations intervening the high-dose generations, pest numbers are sufficiently low from an economic or health perspective, model results suggest that populations can be suppressed longer by abstaining altogether from pesticide application in the low-dose generations.

After 25 treatments when R is dominant ($k=0$), the pesticide is no longer effective at suppressing a pest population for any combination of dosages. When R is intermediate between dominant and recessive, a population can be controlled by the pesticide for a longer time than if R were dominant, but the pattern of control versus first and second doses is similar to when R is completely dominant.

The effect of different dose combinations on the evolution of quantitative resistance is similar to the pattern of the effect of doses on pest population size (figure 10(A)). Using first or second low doses that are below 700 g/ha results in high rates of pest survival after 20 treatments as a result of quantitative resistance. Although this specific value of a cutoff of 700 g/ha results from our categorization of resistance as when the quantitative means is at least 600 g/ha, the general pattern holds no matter how you define quantitative resistance: low doses allow a gradual buildup of resistance, but high doses do not.

This is exactly the opposite to the pattern of major gene resistance (figure 10(B and C)). After 20 treatments, a recessive, major allele conferring resistance completely replaces a dominant susceptible allele only for doses above 1200 g/ha, since mortality of susceptibles must be 100% for resistance to be prevalent within 20 treatments (figure 10(B)). A mere 2.5% survival rate of Rr and rr susceptibles to a dose of 1100 g/ha is

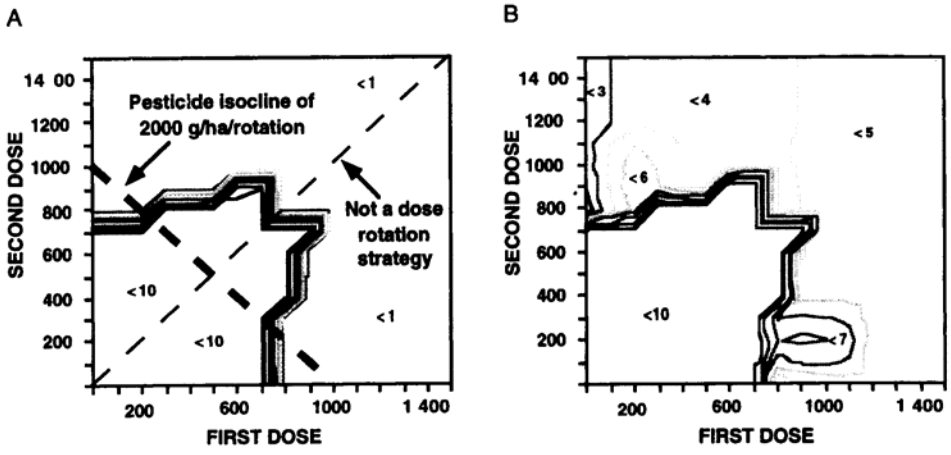

Figure 9. Contour plot of the predicted size of the pest population ($\times 10^{-5}$) versus alternating first and second doses applied in a rotation of two (A) after 20 generations when the major resistance allele R *is recessive or after 15 generations when* R *is dominant; and (B) after 20 generations when* R *is dominant. The heavy dashed line indicates an isocline of the total pesticide applied per rotation. Combinations of first and second doses that result in the smallest population (less than 10^5 pests in (A) and less than 3×10^5 in (B)) while using the least pesticide (intersected by the isocline parallel to the heavy dashed line lying closest to the origin) can be interpreted as the optimal doses to keep pest numbers low over the long term. In part (A) such an optimal (first, second) dose combination is (800,0) or 0,800), and in part B (0,800).*

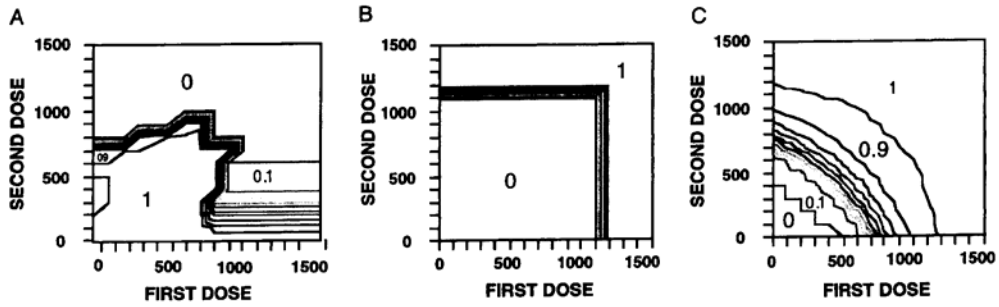

Figure 10. (A) Contour plot of the fraction of the population with quantitative mean resistance greater than 600 g/ha after 20 generations versus first and second doses, alternating in rotation of two as in figure 9; (B) frequency p_{20} of major resistance allele R *after 20 generations versus first and second dose if* R *is recessive; and (C) p_{20} if* R *is dominant.*

sufficient to stave off major gene resistance when the *R* allele is recessive. A dominant, major, resistance allele also occurs at higher frequency after 20 treatments when higher doses are used (figure 10(C)). Since selection can act on heterozygotes, however, allele frequency may be intermediate at moderate doses, compared with the sudden switch from susceptible to resistant in the recessive case.

Combining quantitative and major gene resistance, we can identify which doses delay resistance the longest (figure 11). For very low doses, less than 500 g/ha, resistance evolution can be delayed for over 20 treatments, but population control is deplorable (figure 9). At the opposite extreme, of very high doses above 1200 g/ha, resistance evolves almost immediately after 2–6 treatments, although pest populations can be suppressed. It is in the area of rotating low doses (less than 500 g/ha) with intermediate to high doses (800–1000 g/ha) that both delay resistance for up to 18 treatments and control pest populations for at least that long, as well as use less pesticide than constant dosing would. In short, the trick of rotations is to use high doses just large enough to keep quantitative resistance from becoming a problem, but still small enough so that major gene resistance does not appear immediately.

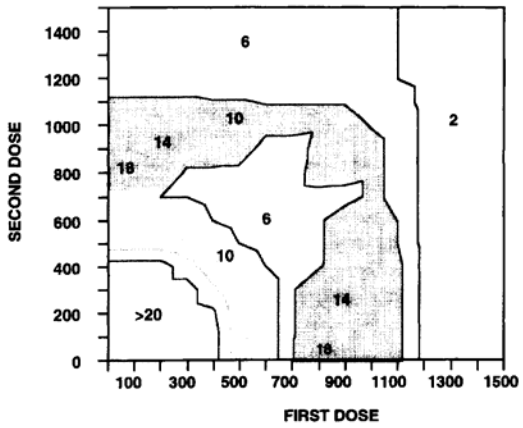

Figure 11. Contour plot of the number of generations until resistance appears versus alternating first and second doses when R *is dominant. When* R *is recessive, the plot looks similar except that in the shaded area resistance may be delayed for more than 20 generations.*

3.1. Choosing the doses

To determine the minimum dosages that must be applied to reset quantitative resistance, one must estimate the mean and the standard deviation of resistance and the size of the pest population. One may gather data on the distribution of resistance by sampling individuals from the population, applying increasing levels of pesticide, and measuring at what dosage each individual dies. If one detects a few individuals with resistance to extremely high doses while the bulk of the population has resistance to only low doses, it is likely that those few have a major allele for resistance, and they should be excluded from the estimation of the distribution of quantitative resistance. Instead, their frequency should be used to estimate the frequency of a major *R* allele.

By using a table of the cumulative normal distribution, for truncation selection a dosage may then be chosen to be a specified number of standard deviations above the mean quantitative resistance. At a dose where the probability that an individual survives is less than the reciprocal of the estimated population size, then quantitative mean resistance can be reset back to that of a pristine, never-treated population. That is, the dose z_d must be chosen so that the area under a standard normal curve (mean = 0, variance=1) above the value $\frac{z-\bar{z}_t}{\sigma_t}$ is less than $1/N(t)$. For a population size of a million individuals exposed to truncation selection, the high dose must be at least 4.8 standard deviations above the mean quantitative resistance of the population for fewer than one in a million to survive. For example, if the mean and standard deviation of resistance are 300 g/ha and 50 g/ha, respectively, the high dose required to reset the quantitative mean resistance is $300+(50\times 4.8)=540$ g/ha. This should be used as a minimum bound on the high dose. If there is uncertainty in the estimates of resistance or population size, or if selection is smooth rather than truncation, then the high dose should be larger than this minimum. Selecting the low dose is easier (see discussion of figure 8(E and F), and may be based on empirical/economic demands of just how low pest numbers need to be suppressed in the low dose years, and the ability

of the pest population to bounce back after the high mortality caused by the high dose.

3.2. Rotation, rate of population growth, and dose

More than one combination of doses and rotations may control a population, and the specific doses chosen depend on the pattern of pest suppression appropriate to a particular situation. When population growth is fast, pest controllers must decide whether they would like moderate control over a longer term, or a high level of control over a shorter term. For example, a high dose of 800 g/ha provides only moderate pest control for about 18 pest generations (figure 12(A)), while a dose of 1000 g/ha provides much better control but resistance renders the toxin ineffective about five generations sooner (figure 12(B)). In contrast, with a slower rate of population growth, the population can be kept small with either 800 or 1000 g/ha (figure 12(A and B)), or by using a longer rotation of four requiring far less pesticide (figure 12(C)).

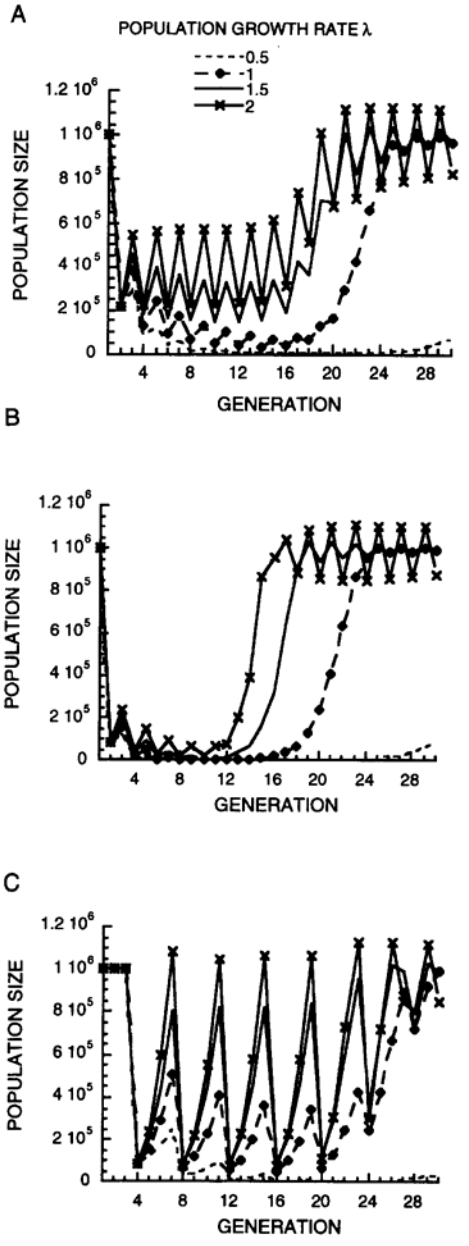

Figure 12. Population size versus generation number for different rates of population growth λ, for (A) alternating doses of 0 and 800 g/ha; (B) alternating doses of 0 and 1000 g/ha; and (C) 0 and 1000 g/ha applied in a rotation of four.

The longest satisfactory series of low doses intervening a high dose depends on the growth rate of the population (λ; figure 12(C)). If the pest population can reproduce quickly, and thus bounce up to high levels of infestation following a high dose, then shorter rotations are necessary. It may be best to monitor population size in the field to guide decisions about when pest numbers are high enough to require a high dose. For example, looking at figure 12(C), one can see that if the high dose had been applied a treatment sooner, the peak population sizes between 8 and 18 treatments would only have reached about half the size. The length of the rotation may be variable if environmental factors such as weather result in variation in population growth rates.

3.3. Modification of the mortality versus dose curve

Using the negative exponential curve (equation (3b)) or the power curve (equation (3c)) instead of the logarithmic curve (equation (3a)) yields virtually identical predictions about which doses best control pest populations and delay resistance the longest (as pictured in figures 9–11). When *R* is dominant, the main difference is that at dose combinations in which the first or second dose is above 1300 g/ha, resistance may be delayed slightly longer (two treatments) using the negative exponential than using the logarithmic curve, while at doses below 1200 g/ha the opposite is true. This occurs because, at lower doses, the strength of selection on major gene resistance is stronger against *rr* susceptibles, while at higher doses it is weaker for the exponential compared with the logarithmic (figure 6). When *R* is recessive, major gene resistance never rises much above its initial frequency using the negative exponential curve, since, even at very high doses, there is some survival of *rr* susceptibles which is sufficient to swamp out an extremely rare resistant *RR* individual. In summary, the results we present using the logarithmic curve are more conservative than the negative exponential curve, predicting a worse situation in terms of resistance evolution. When selection against *rr* is a power function of the dose (equation (3c)) the results are also qualitatively and quantitatively similar to the logarithmic case.

3.4. Initial frequency of major gene

If the frequency of the major allele *R* in a pristine population, p_0, is greater than the 10^{-6} assumed in the preceding results, the qualitative pattern that dosage rotations control populations using less pesticide and delay resistance longer than constant doses still holds. However, resistance evolves faster, no matter the dosage applied, so that population control by the pesticide does not work for as many treatments. For example, for *R*

partially or fully dominant, if $p_0 = 10^{-3}$ and $k = 0$, resistance evolves up to eight treatments sooner than if $p_0 = 10^{-6}$, and the pattern of population control by different doses pictured in figure 8(B) illustrates the situation after 10 treatments rather than after 20 treatments. After 15 treatments, no dose combinations keep pest numbers below the ecological carrying capacity. If R is recessive, resistance appears about a treatment sooner with $p_o = 10^{-3}$ than if $p_0 = 10^{-6}$, and population control (like that pictured in figure 8(A)) begins to break down for extremely high doses (first or second doses above 1200 g/ha) as major gene resistance evolves.

3.5. *Heritability*

The greater the heritability of quantitative resistance, the faster the rise in quantitative mean resistance and the larger the high dose required to control the pest population and to reset the mean (figure 13(A)). This increase in the minimum requirement for the high dose causes major gene resistance to appear more quickly, and pesticide population suppression to fail sooner. But for maximum heritability as high as $h^2_{max} = 0.8$ in equation (14), resistance may be delayed for 10 treatments using dose rotations with a high dose of 1000 g/ha alternated with a generation in which no pesticide is applied, which is substantially longer than the appearance of resistance after only three treatments using a dose of 500 g/ha in every generation.

Even with a high heritability of $h^2_{max} = 0.8$, rotating low and high doses is still the most pesticide-efficient way to keep the population at the smallest possible size after a large number of treatments (figure 13(B)). The most extreme rotation of alternating generations in which no dose is applied with high dose applications is predicted to result in the smallest population after 20 treatments. The results using a heritability function that changes as a result of linkage disequilibrium (equations (12) and (13)) are very similar to the case in which heritability is a function of the mean resistance of the population: higher initial heritability or lower environmental variance requires larger high doses to reset the mean quantitative resistance.

3.6. *Smooth selection*

As the shape of the selection curve of quantitative resistance becomes more gradual (increasing σ_s in equation (7)), the dose required to reset the quantitative resistance and to control pest numbers increases (figure 14). This is because some individuals survive whose resistance is beneath the dose applied. Higher doses, however, mean that major gene resistance evolves sooner. Nevertheless, the qualitative prediction holds with both truncation and smooth selection that dosage rotation is a more pesticide-efficient strategy to control pests than constant dosages, as visualized by a contour plot like figure 9 but with $\sigma_s = 110$ g/ha. It is not pictured here since it looks much the same as figure 9 with concave contours, except the minimum second dose to keep pest numbers below 300 000 is pushed up from 800 g/ha to 1100 g/ha.

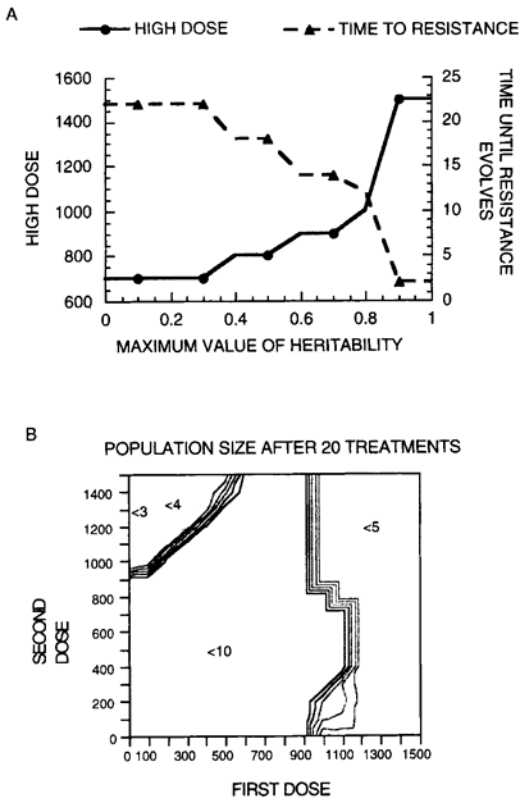

Figure 13. (A) Larger high doses (solid line; alternating with generations of no pesticide application) are required to reset quantitative mean resistance as h^2_{max} increases, with the result that major gene resistance, and thus the total resistance of the population, cannot be delayed as long (dashed line). (B) When $h^2_{max}=0.8$, a plot like that in figure 9(B) (with $h^2_{max}=0.5$) shows that the optimal dose combination to suppress pest populations as long as 20 generations is increased to (0,1000), and that pest numbers are high across more dose combinations when $h^2_{max}=0.8$ than when $h^2_{max}=0.5$.

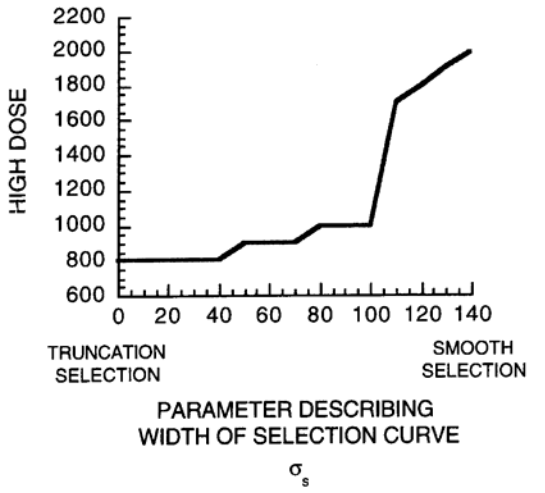

Figure 14. The minimum value of the high dose must increase with σ_s (the smoothness of selection on quantitative resistance) to minimize pest numbers after 20 generations. The high dose is alternated with generations of no pesticide application.

4. Discussion

Model results predict that dosage rotation can suppress pest populations and delay resistance longer using less pesticide than constant doses. This prediction holds whether the major allele for resistance is dominant or recessive, for variations on the functional form of the dose–response curve, whether heritability changes due to linkage disequilibrium or to the mean level of resistance, for different magnitudes of heritability, and for both truncation and smooth selection.

Rotations work especially well when the major resistance allele is recessive since the periodic high doses may be large enough to delay quantitative resistance but major gene resistance still takes very long to become problematic. With a partially or fully dominant allele, selection on heterozygotes for resistance results in a faster increase in major gene resistance than in the recessive case, but a rotational strategy is advantageous, nevertheless, as a consequence of the suppression of quantitative resistance. In most of our results we assumed the more conservative case of a dominant allele.

Heritability of pesticide resistance or tolerance has been measured to be about 0.2 (Tabashnik and Cushing, 1989; Tabashnik, 1992), although values as high as 0.66 for resistance (Tabashnik, 1992) and 0.71–0.85 for tolerance (Firko and Hayes, 1991; Head *et al.*, 1995) have been found. Higher heritability of quantitative resistance requires larger high doses to delay the evolution of resistance and to control a pest population. Similarly, as the probability of survival versus the level of quantitative resistance proceeds from a truncated to a smooth selection curve (e.g. due to uneven application), larger high doses are required.

We have modelled a worst case scenario by ignoring fitness costs often, but not always, associated with resistance (Bergelson and Purrington, 1996). Including a cost in terms of reduced reproduction or survival of resistant individuals in the absence of pesticide or drug treatment or with low doses would slow the evolution of resistance. However, these costs are often very low, so that we feel it is prudent to plan pest management strategies assuming there are no costs to resistance.

The particular application schedule that is best for a particular pest could be selected by using a computer program such as the one we have written (see Section 2.9), and inputting parameter values from the field (mean level of quantitative resistance, variance in quantitative resistance, frequency and dominance of major allele for resistance, etc.). If parameters are unknown, then dosage strategies could be based on estimates of worst case scenarios.

Cancer researchers have found higher rates of mutation, and hence evolutionary change, in tumours (Hartwell and Kastan, 1994; Loeb, 1997). Rapid evolution that leads to resistance to chemotherapy confounds efforts to kill metastic cells (Goldie and Coldman, 1985; Stankovic *et al.*, 1992). Low and then increasing doses have been shown to select for quantitative resistance from gene amplification (Melguizo *et al.*, 1994; Rath *et al.*, 1984; Schimke, 1984). Resistance may also evolve by a single mutational event (e.g. pleiotropic drug resistance; Ling, 1982), potentially favoured by high doses. High initial doses have been suggested to delay the evolution of quantitative resistance by decreasing population size and making incremental increases in resistance unlikely (Hokanson *et al.*, 1986; Coldman and Goldie, 1987; Harnevo and Agur, 1991, 1992, 1993; Panetta, 1998). This proposal to vary doses is similar to ours. Rotating doses may be a more feasible alternative, as an initial high dosage may be lethal to some patients. The initial low doses would better show contraindications of a drug, without lethality, and the subsequent high doses could be selected based on the patient's response.

Our results suggest several practices pest controllers should aim for to facilitate the success of dosage rotations. Although imperfect practices may not render rotations unsuccessful, an effort to follow them is likely to result in better pest control. They are:

(1) Dosage rotations should be applied in concert over the total area covered by a crop across which pests can migrate. Otherwise, intermediate dosage applications may fail to reset the mean quantitative resistance as individuals with quantitative resistance may migrate in after the treatment and mate with individuals with major gene resistance.

(2) Pesticides should be applied as evenly as possible and at a time when all of the pest population is most susceptible to the toxin. This will keep selection for resistance sharp, and more similar to truncation selection than to smooth selection. Lower pesticide doses will successfully delay resistance for longer durations under such circumstances.

(3) Dosage rotation depends on the ability of the intermediate dose to reset the quantitative mean resistance back to a low value. Thus, all efforts should be made to discourage refuges, such as seed banks, where quantitative resistance can be sequestered by individuals protected from the intermediate dose. An example of one such effort is the use of minimum tillage agriculture. As researchers and farmers are often finding long term minimum tillage to be counter-productive, it might be advisable to use minimum tillage in the seasons where low pesticide doses are used and plough only when an intermediate dose of the pesticide is applied. This would bring individuals with major gene but not quantitative resistance into the seedbank. In addition, at some times the refuge may protect fewer individuals than at other times, during which pesticides or drugs should be applied.

We encourage field tests of our models for weeds, insects, fungi, and other pathogens, for it may be easier to delay the evolution of resistance than to find alternative pesticides once resistance is rampant. Once resistance has appeared somewhere, it is actually easier to perform such experiments. One can mix the resistant pests with sensitive ones such that one begins an experiment with higher frequencies of resistance than appear in pristine locations, allowing results to be seen in fewer seasons of experimentation. It would be even more interesting when a pest has evolved major gene resistance in one area and quantitative in another for one to mix both with susceptibles and then perform such experiments. In the future, we plan to model how a population composed of a mix of resistant residents and a substantial influx of susceptible migrants in each generation, or how the presence of a refuge such as a seedbank impacts the effectiveness of the dosage rotation strategy to delay the evolution of resistance.

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Appendix A: Survival and mean resistance before reproduction for $z<z_d$ under smooth selection

To compute the mean survival for individuals with $z<z_d$ we substitute Equations (4) and (7) into Equation (5), yielding

$$\bar{w}_{q,smooth}(z_d|<z_d)=\int_{-\infty}^{z_d} e^{-\frac{1}{2}(\frac{z-z_d}{\sigma_s})^2}\frac{1}{\sigma\sqrt{2\pi}}e^{-\frac{1}{2}(\frac{z-\bar{z}_t}{\sigma})^2}dz \tag{A1}$$

Completing the square in the exponential term and simplifiying

$$\bar{w}_{q,\,smooth}(z_d|z<z_d)=m\int_{-\infty}^{z_d}\frac{1}{\sigma'\sqrt{2\pi}}e^{-\frac{1}{2}(\frac{z-\bar{z}_t}{\sigma'})^2}dz \tag{A2}$$

where

$$\sigma'=\frac{1}{(\frac{1}{\sigma^2}+\frac{1}{{\sigma_s}^2})}\text{ and }\bar{z}_t=\frac{(\bar{z}_t{\sigma_s}^2+z_d\sigma^2)}{{\sigma_s}^2+\sigma^2} \tag{A3}$$

and

$$m=\frac{\sigma'}{\sigma}\exp(\frac{\bar{z}_t{}^2({\sigma_s}^2+\sigma^2)-(\bar{z}_t{}^2{\sigma_s}^2+z_d{}^2\sigma^2)}{2{\sigma_s}^2\sigma^2} \tag{A4}$$

The new mean after selection and before reproduction of individuals with resistance $z<z_d$ is

$$z_{w,z<z_d}=\frac{m\int_{-\infty}^{z_d} z\frac{1}{\sigma'\sqrt{2\pi}}e^{-\frac{1}{2}(\frac{z-\bar{z}_t}{\sigma'})^2}dz}{\bar{w}_{q,smooth}(z_d|z<z_d)} \tag{A5}$$

The numerator may be simplified by substituting and integrating as in Equations (B2) and (B3), resulting in

$$z_{w,z<z_d}=\frac{k[-\sigma'\frac{1}{\sqrt{2\pi}}e^{-\frac{1}{2}(\frac{z_d-\bar{z}_t}{\sigma'})^2}+\bar{z}_t{}'\bar{w}_{q,smooth}(z_d|z<z_d)]}{\bar{w}_{q,smooth}(z_d|z<z_d)} \tag{A6}$$

Survival for individuals with $z\geqslant z_d$ is $\bar{w}_{q,trunc}(z_d)$, and their mean $\bar{z}_{w,z\geqslant z_d}$ after selection is also the same as the mean under truncation selection, given by Equation (B3). The mean after selection and before reproduction is the sum of the means of individuals with $z<z_d$ and individuals with $z\geqslant z_d$, weighted by their survivals:

$$\bar{z}_w=\frac{\bar{z}_{w,z<z_d}\bar{w}_{q,smooth}(z_d|z<z_d)+\bar{z}_{w,z\geq z_d}\bar{w}_{q,trunc}(z_d)}{\bar{w}_{q,smooth}(z_d)} \tag{A7}$$

Appendix B: Derivation of the mean quantitative resistance after selection and before reproduction under truncation selection

Substituting equations (4), (5) and (6) into equation (8) to find $\bar{z}_{tw}$ after truncation selection but before reproduction results in

$$\bar{z}_{tw}=\frac{\int_{-z_d}^{\infty} z\frac{1}{\sigma\sqrt{2\pi}}\exp\left(\frac{-(z-\bar{z}_t)^2}{2\sigma^2}\right)dz}{\int_{z_d}^{\infty}\frac{1}{\sigma\sqrt{2\pi}}\exp\left(\frac{-(z-\bar{z}_t)^2}{2\sigma^2}\right)dz} \tag{B1}$$

The denominator can be evaluated by substituting $u=\frac{z-\bar{z}_t}{\sigma}$ and using an algebraic approximation (Abramowitz and Stegun, 1965) for the cumulative Gaussian distribution. To evaluate the numerator, once again the variables are substituted $u=\frac{z-\bar{z}_t}{\sigma}$ so that $z=\sigma u+\bar{z}_t$ and $dz=\sigma du$. Then

$$\int_{z_d}^{\infty} z\frac{1}{\sigma\sqrt{2\pi}}e^{-\frac{1}{2}(\frac{z-\bar{z}_t}{\sigma})^2}dz=$$

$$\sigma\int_{\frac{z_d-\bar{z}_t}{\sigma}}^{\infty} u\frac{1}{\sqrt{2\pi}}e^{-\frac{1}{2}u^2}du+\bar{z}_t\int_{\frac{z_d-\bar{z}_t}{\sigma}}^{\infty}\frac{1}{\sqrt{2\pi}}e^{-\frac{1}{2}u^2}du \tag{B2}$$

The second term in equation (B2) is $\bar{z}_t w_{q,trunc}(z_d)$. The first integral in equation (B2) can be evaluated by an integration by parts. In summary,

$$\bar{z}_{tw}=\frac{\sigma\frac{1}{\sqrt{2\pi}}e^{-\frac{1}{2}(\frac{z_d-\bar{z}_t}{\sigma})^2}+\bar{z}_t w_{q,trunc}(z_d)}{w_{q,trunc}(z_d)} \tag{B3}.$$