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Strategies to Delay the Evolution of Resistance in Pests: Dose Rotations and Induced Plant Defenses

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Summary

The evolution of pesticide and drug resistance plagues agriculture and medicine worldwide. Resistance may stem from a single major gene or from quantitative sources, and both have been observed in the same species. The genetic basis of resistance plays a key role in treatment, as low doses facilitate the evolution of quantitative resistance, while high doses select for rapid appearance of major gene resistance. When crop rotation or pesticide rotation is unfeasible (e.g. orchard crops or pest cross-resistance to multiple toxins), we propose and model a strategy of rotating low and high doses in alternate treatments. This strategy should delay resistance and extend pest control longer than using a series of constant low or high doses. The model framework was modified to investigate whether inducible (vs constitutive) defense may also slow the evolution of pest resistance to plant secondary compounds, with possible implications for genetic engineering of pest-resistant crops.

Key words: pesticide resistance, drug resistance, evolution of quantitative characters, monogene resistance, dose strategies, inducible defence

Introduction

Pesticide and drug resistance present an escalating problem worldwide. The fallback that

alternative toxins exist, or the hope that they will be discovered by the time that resistance evolves to the currently used pesticides, may give false confidence that resistance is not a major worry. However, some pests seem to have the ability to rapidly evolve resistance to every new pesticide (e.g. *Echinochloa* spp. in rice, the Colorado potato beetle, and ryegrass species in wheat, Gressel et al. 1996). Thus, it is prudent to attempt to delay the appearance of resistance rather than to depend on remediation after resistance is already rampant.

The genetic mechanism responsible for resistance implicates the dosage strategy most likely to delay 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 (Via, 1986, Shaw, 1989, Caretto *et al*. 1994). In contrast, high doses applied from the start prevent quantitative resistance from accumulating because of the improbability that a large number of resistant alleles required for resistance to the high dose will initially be found in a single individual. Major gene resistance, however, 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 models (Georghiou & Taylor 1977; Gressel & Segel, 1978).

Some organisms have evolved resistance by both mono- and quantitative genetic mechanisms (Raymond, *et al*. 1989; Devonshire & Field, 1991; Lande, 1983; Crow, 1957; Galun & Khush, 1980; Putwain *et al*., 1982) and some organisms have evolved one or the other due to different pesticide or drug regimes (Gressel, 1995a; McKenzie *et al*., 1992; Putwain *et al*., 1982). Major monogene resistance had been the prevalent cause of pesticide resistance until farmers started reducing doses (Gressel, 1995b; Gressel *et al*., 1996), a problem likely to escalate as a result of current environmental and economic pressures to reduce pesticide use.

Ideally, one would opt to rotate crops or pesticides, and we advocate such rotations as better all around management strategies. However, some crops cannot be easily rotated (e.g. wheat due to extreme climate or soil conditions, or orchards) and no alternative pesticides exist to which there is no cross-resistance. In these situations, we argue for a preventive tactic of rotating low and intermediate doses to delay the appearance of resistance (Gressel *et al*., 1996). 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. Monogenic resistance emerges more slowly with the moderate selection pressure of rotating doses than if exclusively intermediate or high doses were applied. The size of the pest population also 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. We developed a model to mathematically test this proposal, incorporating quantitative evolution, major gene evolution, and pest population dynamics, which was described in detail elsewhere (Gardner *et al*., 1998).

The strategy of applying different pesticide doses in each pest generation is analogous to the plastic variation in toxin production in response to herbivory used by some plants, called inducible defenses (Karban & Baldwin, 1997). Inducible defenses may impose lower selection pressure on pest populations

than constitutive defense, and therefore delay resistance evolution. Therefore, we used the same mathematical framework to ask whether or not inducible defense could suppress pest populations longer than constitutive toxin production. When modeling the evolution of pest resistance to inducible defense, we assumed that no pesticides are applied. The implications of this are two-fold: 1) natural selection has favored strategies (i.e., inducible defense) which minimize the resistance evolution of pests, and 2) by genetically engineering plants to be constitutively defended we are increasing the probability that pests evolve resistance.

Materials and Methods

In the model, after the pesticide is applied, resistant individuals survive and mate, producing offspring with a Gaussian distribution of quantitative resistance. After each generation of toxin application, the mean quantitative resistance shifts to a higher level that depends on the dose of the pesticide applied. We model the evolution of quantitative resistance according to the standard methods of population genetics (Falconer, 1989).

The major gene conferring resistance (the allele R) also increases in frequency at a rate determined by the relative fitnesses of the genotypes RR, rR and rr under a given toxin dose according to standard Mendelian genetic models (Crow, 1986). Individuals may survive the toxin as a result of either quantitative or major monogene resistance, or both. Alternatively, they may survive because they receive a dose of toxin lower than that intended, due to a number of possible factors. For example, field sprays cannot be applied as evenly as in the laboratory, some individuals may be sheltered by leaves or clods of dirt, and pests of different ages may have different levels of natural resistance. The total fraction of individuals which survive a dose z_d and reproduce is $w(z_d)$.

The pest population size $N(t)$ in generation t is density dependent, following the discrete analogue of logistic growth

$$N(t+1) = \{1 + l[1 - N(t)/N_{\max}]\} N(t) w(z_d) \qquad (1)$$

where $N_{max} = 10^6$ is the maximum size (carrying capacity) of the pest population and l is the per generation rate of increase when then population size is far below the carrying capacity. In the modeling of pesticide resistance, we assumed that $\lambda=1$ in the results presented here.

If an intermediate dose is applied to a population with a low level of quantitative resistance, it is highly unlikely that a single individual possesses a sufficient level of quantitative resistance to survive (i.e. the fraction surviving due to quantitative resistance is less than $1/N(t)$). Individuals that have accumulated a few mutations of small effect are killed before they can produce offspring that are resistant to the intermediate dose applied. Thus, repopulating individuals must migrate from an outside, pristine population or from individuals with monogenic resistance. Therefore, the mean level of quantitative resistance is reset to its low initial value by the application of a sufficiently high intermediate dose. We described the methods of modeling quantitative and monogenic evolution using full mathematical rigour in Gardner *et al*. (1998). Parameter values were chosen based on the system of *Lolium* (ryegrass) as a pest in wheat fields, controlled by spraying the herbicide diclofop-methyl. In the results presented, the major gene was assumed to have additive effects on resistance (i.e., midway between a dominant and a recessive allele).

Qualitatively, the main results hold regardless of the dominance of the major allele. Heritability of quantitative resistance was assumed to increase in the initial stages of selection, and then to decline as resistance alleles became fixed. Other assumptions about how heritability might change with selection did not alter the conclusions of the model.

We used the same model framework described above to address the question of whether or not induced host plant production of secondary compounds might delay the appearance of resistant pest populations longer than would constitutive toxin production. For constitutive defense, we assumed that pests were exposed to plant secondary compounds in every pest generation, regardless of the pest population size. For inducible defense, we modeled that, initially, at low pest concentrations, the pests experience no toxin. Once the pest population size grows in excess of a threshold size of N_{induce}, they induce the host plants to produce secondary compounds which reduce $w(z_d)$ and thus pest population growth by an amount that depends on the level z_d of toxin production. If the pest population size drops below N_{induce}, then after q pest generations, host plants cease to produce toxin. We examined how both the toxin concentration z_d and the persistence of defense q after pest population reduction affect the duration until pests evolve resistance to host plant toxins. Based on experimental data from a system of cucumber (*Cucumis sativus*) host plants and spider mite (*Tetranychus urticae*) pests, we used λ=0.3 (Agrawal, unpublished manuscript). We assumed N_{max}=1000, and N_{induce}=100. A pest population was said to be resistant if either the mean level of qualitative resistance was three times higher than in an untreated population or if the frequency of the major gene for resistance reached a frequency of at least 30%.

Results

Pesticide Resistance and Dose Rotations

The patterns of evolution of resistance (parts A and B) and the change in size of the pest population in response to the alternative strategies of applying a constant dose every pest generation (part A), or a dose rotation in alternate generations (part B), are modeled in Fig. 1.

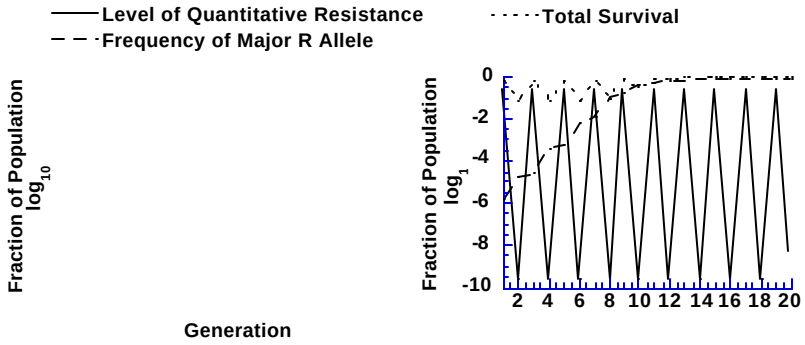

Fig. 1. Survival due to quantitative resistance (continuous line), the frequency of the major allele for resistance (dashed line) and the total fraction of the population surviving (dotted line) for A) a constant dose of 700 g/ha, and B) a rotating dose strategy of 300 g/ha and 1000 g/ha in alternating pest generations. C) Pest population size under the constant dose

strategy shown in part A (dashed line) and the rotating dose strategy shown in part B (continuous line). Model results indicate that dose rotation delays resistance and controls pests longer than constant doses.

In the modeled data in Fig. 1A, survival resulting from the evolution of quantitative resistance increases quickly. A high fraction of the population survives since the dose of 700 g/ha exerts only weak selection for resistance, and therefore the rise of the frequency of the major allele conferring resistance is slow. In modeled data in Fig. 1B, many individuals survive due to quantitative resistance in the generations the very low dose is used, but none survive the high doses. Major gene resistance increases slightly more rapidly than in part A, but not as quickly as the mere 6-7 generations it would take if a constant high dose of 1000 g/ha had been used. Consequently, the total level of resistance from both major gene and quantitative sources arises more slowly using a dose rotation, and pest population growth may be suppressed for approximately 18 generations longer than could be achieved using a constant dose in every generation. Moreover, over the course of 10 pest generations the constant scheme uses a total of 7000 g/ha of pesticide, while the dose rotation uses only 6500 g/ha.

The implications of Induced versus Constitutive Host Defense

We compared pest population growth and evolution of resistance to host defense on plants that constitutively produce enough toxin to reduce the fitness of unselected pests by 40% (1- $w(z_d)$), to that of plants with induced defenses that reduce pest fitness by 60% (Fig. 2).

Quantitative resistance rises rapidly when plants continuously defend themselves at a low rate, regardless of pest density, since toxin production is relatively low and individual pests rapidly accumulate many alleles of small effect to resist host defense (Fig 2A). In the scenario plotted major gene resistance takes longer to appear than quantitative resistance. Still, this would not be the case if constitutive toxin production were higher, at a level that would reduce pest fitness by 60% (not shown). Pests suffer the reduced fitness for only for a few generations after host defenses are activated (Fig. 2B). During the periods when the defenses are active, quantitative resistance cannot evolve if hosts produce a sufficient level of toxin to preclude survival of partially resistant pests. Plants having inducible defenses can delay the appearance of high levels of major gene resistance much longer than plants with constitutive defenses, as there is no selection pressure when the pest population is small.

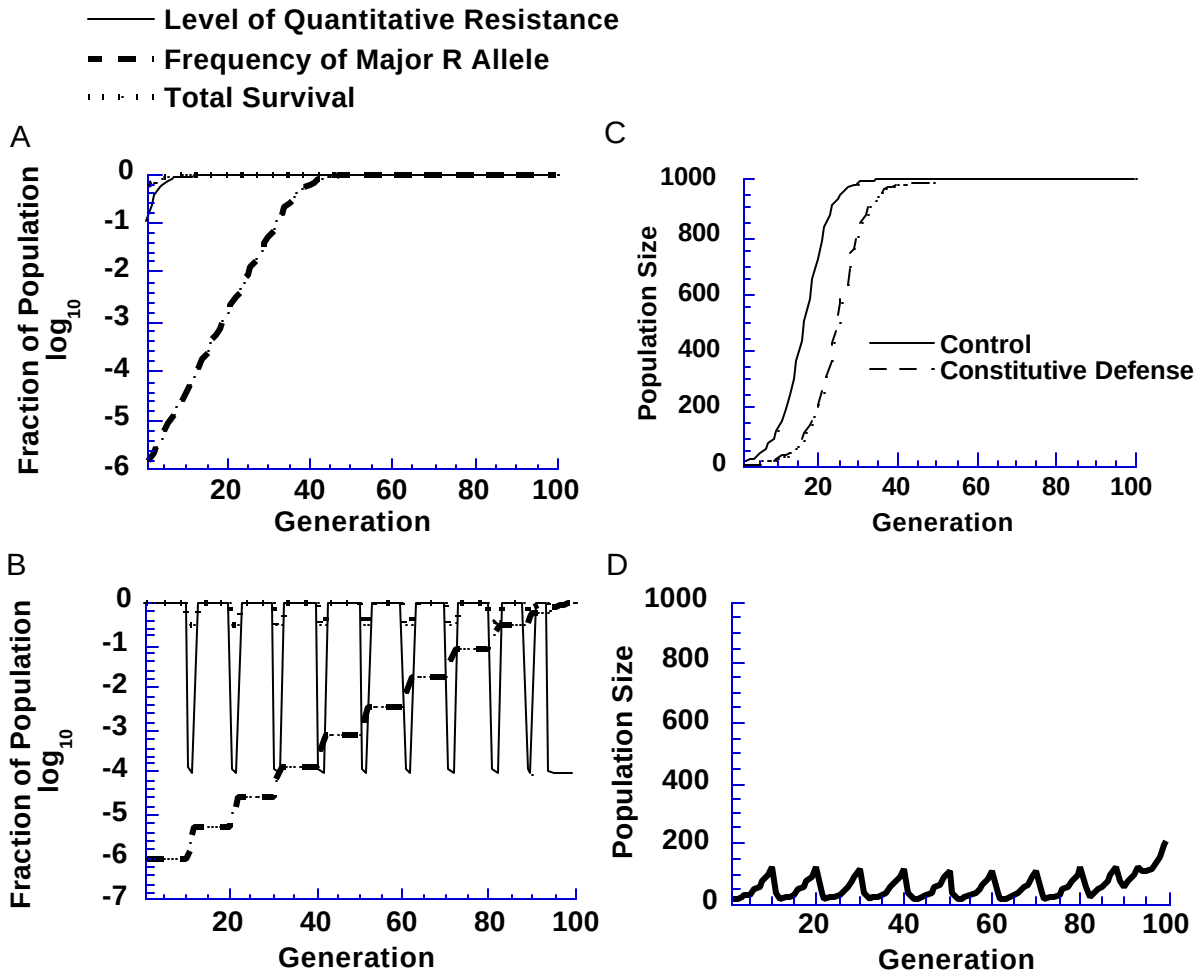

Fig. 2. Survival due to quantitative resistance (continuous line), the frequency of the major allele for resistance (dashed line) and the total fraction of the population surviving (dotted line) for pests on hosts with A) constitutive, low levels of defense (see text) and B) induced defense at a higher level. The legend above part A refers to both parts A and B. Pest population size for pests on C) hosts with constitutive defense as in part A, and D) induced defense, as in part B. Since resistance takes longer to evolve with induced than with constitutive defense, pest numbers remain low for more generations.

The model predicts that pest populations on plants with constitutive defenses may be suppressed longer than those on control plants incapable of defense (Fig. 2C). Induced host defense, however, may keep pest numbers low for tens of pest generations longer than constitutive defense (Fig. 2D), although during the initial period when constitutive defense still works (first 10-20 pest generations), pest numbers may be lower with constitutive than with induced resistance. Moreover, model calculations indicate that the total amount of toxin plants must produce is about 2.3 times higher for the constitutive than for the induced defense scenarios pictured in Fig. 2.

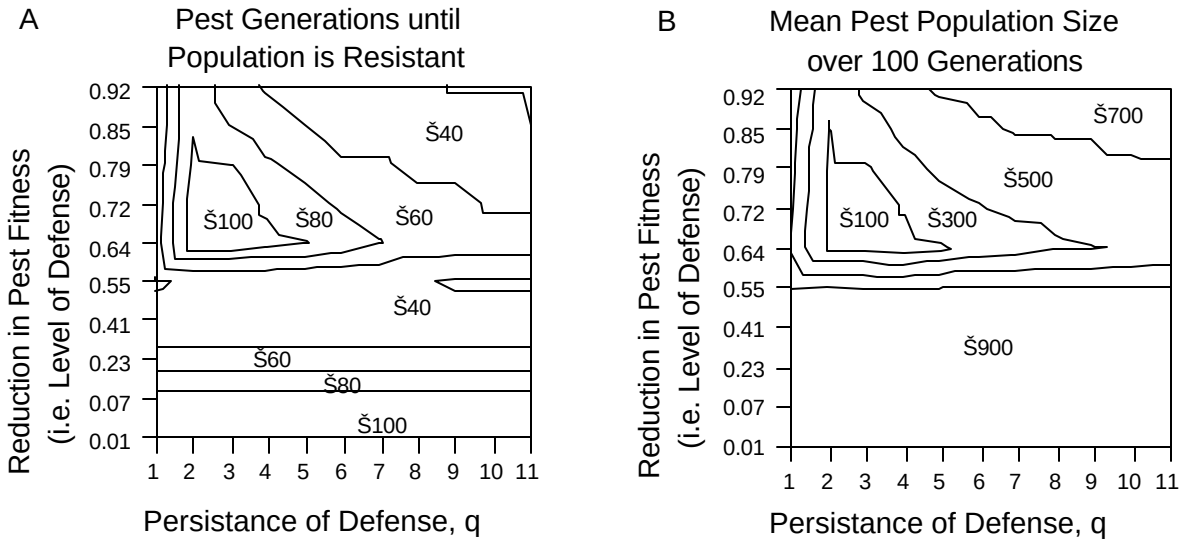

Fig. 3. Contour plots showing the joint effects of the level of defense and the persistence of defense after induction on A) the time until the population is resistant (see text) and B) the average pest population size over 100 pest generations. Resistance may be delayed longer and the mean population size is smaller if host defense after induction is at a level to reduce pest fitness by about 60-80% and is maintained for only 2-4 pest generations after induction.

In addition to comparing constitutive versus induced defense, we also used the model to examine quantitative differences in induction strategies. Contour plots illustrate model predictions that the delay until a pest population becomes resistant (quantitative and major gene combined) peaks for a narrow range of combinations of the level of defense (1- $w(z_d)$) and the persistence of defense (q) (Fig. 3). Ideally, reduction in pest fitness of about 60-80% combined with defense that continues for 2-4 pest generations after pest numbers reach the threshold for induction result in the longest delay until resistance becomes rampant (Fig. 3A). Prolonged efficacy of defense from these combinations results in lower pest population sizes (averaged over 100 pest generations, Fig. 3B). If toxin production stays high for more than 5 pest generations following the initial induction and reduction of pest numbers, resistance soon evolves and pest numbers rise.

Discussion

Model results predict that dosage rotation can suppress pest populations and delay resistance longer using less pesticide than constant doses. Calculations also indicate that induced host defense may control pest populations longer than constitutive defense. Pesticide application strategies, natural host plant defenses, and genetically engineered crops to withstand pest attack all must contend with problems of pest evolution of resistance by quantitative and major monogenic mechanisms. Varying toxin levels depending on the size of the pest population may be a cost effective way to prolong the success of pest control while being economically, energetically, and/or environmentally advantageous. Farmers may well learn from the

plastic strategies employed by plants with induced resistance: monitoring pest numbers and only applying pesticides when numbers are high. If gains are measured over less than 15 pest generations the benefits over constant pesticide application may not be detected, and the effort of monitoring may not seem worth the extra effort. However, over the longer term, model predictions suggest that dose rotations or dose variation in response to pest density may really pay off.

If the biosynthesis of secondary compounds requires substantial amounts of carbohydrates or other nutrients, induced defenses may provide a real savings in production costs, especially when they have a short half life. However, the energy costs of toxin production may be minimal or nonexistent in some cases (Mole, 1994), although there is contention about such costs (Herms & Mattson, 1992). Delayed pest evolution of resistance and lower average pest numbers over the long term may be important factors in favoring induced over constitutive plant defenses (Agrawal & Karban, 1998).

One of us (J. Gressel) is currently planning field tests of the dose rotation strategy using ryegrass. 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.

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