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# Modelling the Effectiveness of Herbicide Rotations and Mixtures as Strategies to Delay or Preclude Resistance[1]

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**Abstract.** Herbicide-resistant populations have evolved only in monoculture and/or monoherbicide conditions at predictable rates for each compound and weed. No populations of triazine-resistant weeds have appeared in corn where rotations of crops and herbicides or herbicide mixtures were used. This is due to the greatly reduced competitive fitness of the resistant individuals, which could be expressed only during rotational cycles, and also to the greater sensitivity of resistant individuals to other herbicides, pests, and control practices ("negative cross-resistance"). The model presented here describes how an understanding of all of these factors can provide strategies to decrease the frequency of the resistant individuals during rotation. Rotations or mixtures may not delay the rate of appearance of resistance to inhibitors of acetolactate synthase (ALS), where the fitness of resistant biotypes is claimed to be near normal. The best way to delay resistance to ALS inhibitors is to use those compounds with less persistence so that the selection pressure will be lowered. Too little is known about the frequency of resistance to other herbicides with target-site resistance–to dinitroanilines, to acetyl CoA carboxylase inhibitors, or to those situations where a single enzyme system confers resistance to a broad spectrum of seemingly unrelated herbicides. **Nomenclature:** Corn, *Zea mays* L.

**Additional index words:** Herbicide resistance, fitness, selection pressure, seedbank dynamics, triazine resistance, metabolic cross resistance.

## INTRODUCTION

Weeds are evolving resistance to different herbicides at different rates. Resistance can be avoided by understanding and analyzing the interacting factors involved in changing a sensitive weed population into a resistant one. These factors, described below, are inserted into models so that the quantitative importance of each factor can be evaluated. The model predictions favorably compare with the case histories of resistance. Newer models are described so that the effectiveness of different weed control strategies can be predicted better.

Weeds in North America have evolved resistant populations to herbicides only where there was monoculture with a single family of herbicides. The only exceptions until recently have been where different herbicides having the same site of action were used (e.g., a rotation of photosystem-II inhibiting herbicides) (17). Resistant-weed populations have evolved in wheat (*Triticum aestivum* L.) monoculture in England and Australia, where high selection pressure herbicides with different sites of action but the same putative mode of degradation are rotated (17, 19, 37, 41). Such metabolic cross resistances likely will evolve elsewhere.

The appearance of resistance in monoculture and/or mono-herbicide usage was described by a simple population model (22, 23) that integrated the following: a) the selection-pressure of the herbicide (based on the rate used, its effectivity with particular weeds, and its persistence); b) the germination dynamics of the weeds (over the season and from the soil seedbank); c) the initial frequency of resistant individuals deriving from natural mutations in the susceptible population; d) the fitness of the evolved resistant biotypes in competition with the wild type under field conditions; and e) the number of generations (seasons) the herbicide was used. This model helps to understand why resistance evolved in monoculture to the high selection pressure *s*-triazines in corn but not to herbicides exerting lower selection pressure, such as the thiocarbamates, chloroacetamides, and phenoxy herbicides, in similar weeds growing in this crop (22, 23).

It is consistent with the model that populations of weeds did not evolve that resist 2,4-D [(2,4-dichlorophenoxy)acetic acid] and MCPA [(4-chloro-2-methylphenoxy)acetic acid] in wheat. The model predicts the inevitably rapid appearance of weed biotypes resistant to the high residual activity sulfonylureas, as well as to chlorotoluron [*N'*-(3-chloro-4-methylphenyl)-*N'N*-di-

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methylurea], diclofop-methyl {methyl ester of (±)-2-[4-(2,4-dichlorophenoxy)phenoxyl]propanoic acid}, and mecoprop [(±)-2-(4-chloro-2-methylphenoxy)propanoic acid)] in weeds of wheat.

Resistant biotypes evolved first in those weeds where the herbicides exert the highest selection pressure. For example, diclofop exerts a higher selection pressure on ryegrass (*Lolium* spp.) species than on wild oat (*Avena* spp.) species. In agronomic terms, this herbicide is more effective on ryegrasses, and ryegrasses indeed have evolved resistance with greater rapidity (41).

Because of a lack of field ecology data, our early model inaccurately predicted evolution of resistance where herbicide mixtures and rotations were used. Vast areas of corn have received herbicide rotations including *s*-triazines for 30 yr, and resistant populations have not appeared. Our early model did not adequately consider the then unknown effects of the extreme lack of fitness in many resistant biotypes in the seasons that triazines were not used. The previous model (22, 23) correctly predicted that mixtures could delay considerably the evolution of resistance, but the lack of field data on selection pressure of mixtures left it to the reader to insert the correct parameters into the equations and the accompanying figures.

A new model (24) considers what happens during the "off" years when a given herbicide is not used and predicts that some high selection pressure herbicides can be used sparingly in rotation, possibly even after resistance has appeared. The data show the urgent need for further research concerning the physiological ecology of resistant weed populations.

## MODELS

The original monoculture model described different possible rates of enrichment of resistant individuals in populations, until the populations were predominantly resistant (22, 23). Different constant proportions of susceptible and resistant plants germinated and survived to the end of the season, and susceptible and resistant individuals had (different) constant seed yields. Resistant individuals initially formed an exceedingly small fraction of the population, certainly a well-warranted assumption during the years of resistance enrichment, until there were sufficient numbers of individuals for resistance to be evident.

Various factors involved in evolution were found (after certain simplifications) to satisfy a simple algebraic equation giving our early model (22, 23). Solving this equation gives the frequency of resistant individuals after $n$ years of treatment.

$$N_n^{(R)} = N_o^{(R)} \left[1 + \frac{f\alpha}{n}\right]^{\bar{n}} \quad [1]$$

The factors included in the original monoculture monoherbicide model equation are the following:

$N_o^{(R)}$ is the very low frequency of resistant individuals in the population before it is exposed to the herbicide. Resistance is sustained in the population in the absence of herbicide by a balance between new mutations to resistance and depletion of a proportion of resistant individuals by their lesser fitness in the absence of selection. This results in a resistance fraction somewhat lower than the mutation frequency. Mutants conferring more fitness than the wild type become the wild type. Mutant fitness can be near neutrality, and the mutants would be found in different proportions at various geographical areas due to genetic "drift." $N_n^{(R)}$ is the proportion of resistance in the population after $n$ seasons of treatments.

$f$, the competitive reproductive fitness, measures the compounded relative robustness of resistants in competition with susceptibles during germination, establishment, growth, pollination, seed production, and survival. By definition, fitness always is measured with resistant and susceptible plants in competition with each other in the absence of herbicide. When they are grown separately, the resistant individuals are often less "productive," but the competitive fitness differential is usually greater (Table 1)[3].

$\alpha$, the selection pressure, is the ratio of the fraction of resistants that abound in the population after a herbicide application during that season to the corresponding fraction of susceptibles. Thus, early or late-germinating susceptible individuals that produce seeds are considered in this "effective-kill", in contrast with the initial weed control usually measured by weed scientists. For example, if the herbicide kills 90% of the susceptibles and none of the resistants, then $\alpha = 10$; if 99% of the susceptibles are controlled and none of the resistants, then $\alpha = 100$. The susceptible individuals would in-

[3]Letters following the symbol # are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 309 W. Clark St., Champaign, IL 61820.

*Table 1.* Lower productivity and competitive fitness of *s*-triazine-resistant biotypes[a].

| Species[b] | Productivity | Competitive fitness (1:1)[c] | Reference |
|---|---|---|---|
| | (Resistant ÷ Susceptible) | | |
| Smooth pigweed | 0.90 | 0.18 | 1 |
| Common groundsel | 0.47 | 0.43 | 28 |
| Common lambsquarters | 0.75 | 0.08 | 52 |
| Lateflowering goosefoot | 1.00 | 1.78 | 52 |
| Rapeseed | 0.76 | 0.28 | 21 |

[a]Productivity is measured by growing resistant and susceptible biotypes separately; competitive fitness is measured by growing them in a mixture. In both cases, seed yield was measured, where "seed" may include fruit or whole flower depending on study cited.

[b]Smooth pigweed (*Amaranthus hybridus* L. # AMACH); common groundsel (*Senecio vulgaris* L. # SENVU); common lambsquarters (*Chenopodium album* # CHEAL); lateflowering goosefoot [*Chenopodium strictum* Roth. var. *glaucophyllum* (Aellen) H. A. Wahl. # CHESG]; rapeseed (*Brassica napus* L.)

[c]Fitness of 1:1 mixture.

clude those missed by sprays (in refuges) and "immigrants" due to seed and pollen influx.

The gene flow due to seeds and pollen usually is minimal, in the range of meters per years when actually measured (2, 30, 43). Immigration of resistant seed can be a problem when there is strong selection pressure in a field. One "pioneer" seed can form a large colony. Immigrating susceptible seed will have little numerical impact because of the larger reservoir of susceptible seed already in the field during the first cycles of selection for resistance.

$\bar{n}$ is the average number of years that a seed remains viable in the seed bank.

The predicted rates of enrichment of resistance are plotted from Equation [1] with different scenarios of selection pressure, seedbank dynamics, fitness, and initial frequency in Figure 1. These various measures of resistance change by a constant factor each year, giving rise to an exponential increase in resistant individuals (Figure 1). Estimated parameter values (that should have come from experimentation) were inserted into the equation to generate the scenarios. They were based on a limited data base, mostly from corollary systems, such as heavy metal tolerance. Research groups have begun to accurately measure weed-herbicide interactions for more precise estimates.

The frequency of resistance in the population starts at a low value and increases by a constant factor each year. In spite of the exponential increase, detecting resistant individuals in a field will be hard until resistance is at a level of 10 to 30% of the population.

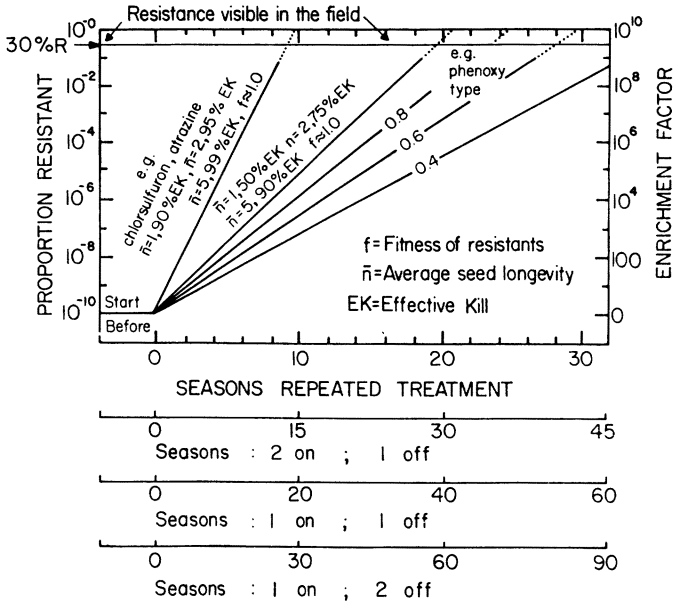


*Figure 1.* Presumed effects of herbicide rotations using the original model. Overall average effect of scenarios with different selection pressures [$\alpha = 10$ = 90% effective kill (EK); $\alpha = 100$ = 99% EK; $\alpha = 2.0$ = 50% EK], seed bank dynamics ($\bar{n}$), the average seed duration in the soil, and *f* differential fitness. The different scales give the different rotational scenarios from monoherbicide to one treatment in three seasons. Calculated from equations in (22, 23). Modified from (24).

**Messages from the model.** *a) The importance of selection pressure.* Selection pressure is the most influential agronomic variable, with the largest effect on the evolution of resistance (Figure 1). For example, the atrazine [6 -chloro -*N*′ -(1 -methylethyl) -1,3,5 -triazine-2,4 -diamine] and chlorsulfuron {2-chloro-*N*-[[(4-methoxy-6-methyl -1,3,5 -triazin -2 -yl)amino]carbonyl]benzenesulfonamide} levels required to kill different weeds vary over more than an order of magnitude. The selection pressure of both herbicides is greatest on annual broadleaf species requiring the lowest rates for weed control. The selection pressure is least for those weeds requiring the highest herbicide levels, i.e., usually the grasses. At a given herbicide rate, both herbicides control broadleaf species better, i.e., selection pressure is lower on grasses.

The first weeds to evolve atrazine-resistant populations were common groundsel, pigweed species, and lambsquarters species. The last to evolve resistance were the grasses, as the model predicted. Various broadleaf weeds already have evolved chlorsulfuron resistance in the field, at the level of the target enzyme. This occurred under repeated selection pressure of this

recently introduced and highly persistent herbicide. It was also easy to select for resistance to this herbicide in the laboratory (7, 26). If these two preferentially broadleaf controlling herbicides were used in conjunction with selective grass-controlling herbicides, their use rates could be decreased and the selection pressure on broadleaf weeds lowered.

Single annual use of herbicides with the greatest persistence always will exert the highest selection pressure (Figure 1). The triazines, dinitroanilines, and sulfonylureas meet this criterion of persistence with season-long control, and resistance has evolved to all these groups (16). 2,4-D and other phenoxy herbicides and thiocarbamates have short biological persistence in the soil, and resistance has not evolved to them. Resistance need not have evolved so rapidly. There are less persistent triazines than atrazine (such as cyanazine) {2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropane-nitrile] and far less persistent sulfonylureas for wheat than chlorsulfuron and metsulfuron {2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-amino]carbonyl]amino]sulfonyl]benzoic acid} (5). If these less persistent analogs had been used, the selection pressure should have been lowered and the evolution of resistant populations delayed.

Paraquat (1,1′-dimethyl-4,4′-bipyridinium ion) resistance has evolved and may seem to contradict the theory's emphasis on persistence, as paraquat immediately loses biological activity upon reacting with soil colloids. The lack of residual persistence was balanced by farmer persistence. Paraquat resistance occurred where this herbicide was used 5 to 10 times during each season in monoherbicide usage.

*b) Seedbank dynamics.* The longer the life in the seedbank, the greater the buffering effect of susceptible seed from previous years, decreasing the rate of evolution of resistance. Common groundsel has evolved triazine resistance in orchards, nurseries, and roadsides where there was no mechanical cultivation but not in cultivated corn fields. The groundsel seed is incorporated into the soil seedbank in such corn fields, where it is viable for many years (54). All groundsel seed falling on undisturbed soil on roadsides or orchards either germinates or dies during the following season (42). Resistance thus evolved where there was the lowest average seed bank life time $\bar{n}$ as predicted. Such information must be considered in formulating strategies for resistance management. Many other species do not have a seedbank under specific agronomic situations, i.e., in minimum-till agriculture where $\bar{n} = 1$.

*c) f, fitness of resistant individuals.* The lack of fitness of resistant individuals can have a strong dampening effect on the evolution of resistance but only when it can be expressed, i.e., when the herbicide is not present. Thus, in monoherbicide culture, the lack of fitness can have little influence with persistent herbicides but could be effective with the less persistent herbicides. This is another reason to avoid persistent compounds, especially in monoculture.

**Resistance to herbicides with the same site of action.** The original model predicted that rotation or mixing of herbicides with the same site of action (and thus similar mode of resistance) will have the same effect as using a single herbicide. This was expected on biochemical grounds as well; plants resistant to atrazine had target-site cross resistance to all triazines, some phenylureas, uracils, etc., all at the same site in Photosystem II. The use of corn/soybean [*Glycine max* (L.)Merr.] or atrazine/metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5-(4*H*)-one] or sulfonylurea/imidazolinone rotations with various crops thus should be contraindicated if the various herbicides are effective on the same spectrum of weeds (17, 18, 23). For the same reason, the genetic engineering of atrazine-resistant soybean for use in monoherbicide culture would be misdirected unless the atrazine usage in corn were to be replaced by other herbicides.

**Cross resistances to herbicides due to degradation.** Metabolic cross resistances to insecticides and drugs having vastly different modes of action are common and are documented to the level of molecular biology (16). Such cross resistances to herbicides with different modes of action are a recent occurrence (17, 18, 27, 37, 41). Rigid ryegrass (*Lolium rigidum* L.), frequently called annual ryegrass, that evolved resistance to diclofop had cross resistance to chlorsulfuron as well as to all other wheat-selective herbicides (27). Blackgrass (*Alopecurus myosuroides* Huds. # ALOMY) that evolved resistance to chlorotoluron was cross resistant to chlorsulfuron, pendimethalin [*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine], and diclofop (37).

Similar metabolic cross resistances to insecticides and drugs often were traced to the evolution of higher levels of nonspecific esterases, hydrolases, or monooxygenases. The resistances in blackgrass and rigid ryegrass can be abolished by adding specific monooxygenase inhibitors along with the herbicide (19, 20, 32, 41). Genetically engineering new modes of herbicide

resistance into wheat also could alleviate the problem (18, 19). Such data, along with the knowledge that wheat seems to have selective resistance to herbicides by mono-oxidations, suggest that these weeds may have evolved a biochemical mimicry, i.e., they have evolved a system similar to wheat to degrade wheat-selective herbicides (17, 18, 19).

## MODELLING ROTATION

The model as shown in Figure 1 does not adequately account for events in the "off years" during rotations if the competitive fitness of the resistant biotype is low. Resistance is shown (Figure 1) to evolve at a fixed rate as a function of the number of generations or seasons a weed was treated with a particular herbicide. This means that if it would take 6 yr for resistance to occur in monoculture corn with atrazine as the sole herbicide, it either would take 9 yr in a corn/corn/wheat (or soybean) rotation where atrazine is used for control 2 of every 3 yr; or 12 yr in a corn/wheat (or soybean) rotation where atrazine is used every other year, or 18 yr in a corn/wheat/soybean rotation where atrazine is used once in 3 yr.

When the model was formulated a decade ago, triazine resistance appeared in areas of the cornbelt where such rotations were used, as there had been 6 to 10 yr of atrazine usage since it was introduced. Yet, resistant populations only appeared in monoculture, monoherbicide corn.

**Mixtures.** Few farmers in the center of the cornbelt grow monoculture corn, unlike areas to the east of the cornbelt where triazine resistance appeared in corn. Cornbelt farmers use rotations and/or mixtures of triazines with chloroacetamide herbicides, which allow the use of less atrazine and thus lower the selection pressure. The chloroacetamides also kill pigweeds (*Amaranthus* spp.) and lambsquarters (*Chenopodium* spp.) as well as grass weeds. Mixtures substantially delay resistance, both according to the model (22) and from field data, although the magnitude of this effect is yet unclear.

The reason for the efficacy of mixtures to delay resistance can be manyfold. The frequency of resistance is lowered to the compounded frequency for each component. If the frequency of resistance to one component is $10^{-7}$ and the other $10^{-8}$, the compounded frequency is $10^{-15}$. The lower rates of each component used decreases the selection pressure for each, adding to the delay in resistance. The fitness to each component is also compounded, which should give low fitness for the individual resistant to both compounds in a mixture. Simulations of this are illustrated in (22, 23). Certain mixtures are not "mixtures" in the sense of delaying resistance: those that act at the same site (e.g., two photosystem II inhibitors) and those that are degraded by the same enzyme system (e.g., possibly all the herbicides used in wheat that are degraded by monooxygenases).

The models for mixtures are described in greater detail, with figures showing scenarios, in (23). Herbicide rotation may be the only strategy remaining to delay the evolution of triazine resistance in corn as the chloroacetamide herbicides used in mixtures for corn are under attack for environmental reasons, and their use is restricted in many areas and forbidden in others.

We have incorporated better data into an updated model, to show how rotation has been a better strategy than previously predicted. The newer data and model emphasize the highly reduced fitnesses of the resistant biotypes, which are of greater magnitude and importance than had initially been expected.

*Lack of fitness of resistant weeds – a major consideration.* The initial model used an average fitness differential for all generations treated (Figure 1). The fitness differential between resistant and susceptible individuals essentially never can become apparent with herbicides such as triazines that give season-long control, as there is no time when the herbicide is not present for this differential to be expressed. Only the resistant biotypes can survive when the herbicide is present. Thus, the fitness differential is unimportant with triazines in monoherbicide culture but seems to be an important factor in delaying resistance to other herbicides with more ephemeral action.

The fitness differential is important when herbicide usage is stopped for a season or more. Resistant biotypes are often more susceptible to some of the herbicides and cultivation procedures used in the rotational years (negative cross resistance). We have modified the model to consider what happens to resistant individuals in the "off" years when the herbicide in question is not used (24).

The resistant individuals, initially present at very low frequencies in the field, must compete with the crop and with resistant members of other weed species. When the herbicide is not present, they must compete with susceptible members of the same and other species

that germinate throughout the year. During the evolution of resistant populations, only intraspecific competition has been considered, except for one study (53). More data are needed from the agro-ecologists on the important of interspecific competition, including that with crops and weeds.

The first studies on competitive fitness were performed by pregerminating seedlings of resistant and susceptible individuals, interplanting them at fixed distances, and allowing them to grow to maturity (9). The yields of the resistant and susceptible biotypes were measured. In almost all cases where this was done, the susceptible individuals outyielded the resistant ones (Table 1).

Most competition experiments have not been made with material that has nuclear isogenicity where resistant and susceptible alleles are in otherwise identical nuclear backgrounds. Nuclear isogenicity is easy to achieve by using reciprocal hybrids when dealing with cytoplasmically inherited triazine resistance. An otherwise identical plastome in such crosses cannot be guaranteed. This eventually may change when plastomes are engineered by site-directed mutagenesis. Repeatedly backcrossed material also provides near-nuclear isogenicity with a large differential in competitive fitness (21) (Table 1). There seems to be no fitness difference between resistant and susceptible individuals of lateflowering goosefoot (53). Lateflowering goosefoot is a slow-growing species, and photosynthetic electron transport probably does not limit its growth.

A few triazine-resistant grasses have been reported to be more productive and competitively fit than the wild type (56), but this must be checked under more rigorous conditions. In general, one must be wary of reports of evolved fitnesses that are better than the wild type. Considering the long periods that species have evolved, the wild type in any given location should have evolved to optimal fitness. Spurious reports of high fitness may result form various interrelated functions: a) fitness was not measured from germination on; b) density dependent functions were not considered; c) reactions to environmental conditions may have differed; d) germination characters and seed bank dormancies may have differed; and e) fitness was not measured under field conditions.

Some mutations to resistance may engender larger losses of fitness than others. Triazine resistance may lead to an extreme case of fitness loss that could be due to many other linked mutations in the chloroplast genome. Triazine resistance may evolve in populations containing a plastome-mutator gene (3). This could explain some of the unfitness of triazine-resistant plants as well as the variabilities of plastid fitness. Any plant with triazine-resistant plastids should have other mutations in its plastids. Deleterious nuclear mutations can be bred or selected out of populations because of chromosomal segregation along with somatic and meiotic recombination (crossing over). This is not as easy with chloroplasts, where recombinations are negligible. Thus, the unfitness in atrazine-resistant plants may not be due to the *psb*A gene mutation, as has been argued on biophysical grounds (31, 34, 47, 49).

Target site mutations or gene amplifications in enzymes present in low quantities may not exert such strong effects on resistance as was found with triazine resistance. Fitness measurements must be performed carefully with resistant weeds as soon as resistant populations appear.

**The measurement of fitness.** As we consider fitness a major factor in delaying resistance, the measurement of fitness is described in detail below.

*a) Measurement from germination.* Weeds produce hundreds to thousands of seeds to replace one plant. Most perish before maturity, many during overwintering, early germination, and establishment. The competition before establishment is probably the fiercest. Competitive fitness studies should be measured at this stage, but this usually has not been done. The simplest way to do this is to plant mixtures of resistant and susceptible seed and to ascertain which plants are resistant and susceptible by a nondestructive test or by using leaf pieces. The seeding should be done at various depths and densities and preferably under field conditions to best mimic the natural environment. As seeds from resistant plants are often smaller than those from sensitive plants, there surely should be a definite competitive disadvantage to resistance when the selecting herbicide is not present.

*b) Density dependence of fitness.* Density dependence of fitness has not been measured adequately. For example, it is not clear why annual bluegrass (*Poa annua* L. # POAAN) biotypes resistant to triazines evolved only in genotypes that were prostrate and not in those that are erect (10). The lack of competition in triazine-treated fields possibly allowed the prostrate types to spread. There are variations in the density dependence of diclofop-resistant rigid ryegrass (27). The resistant plants were more fit than the sensitive ones under

sparse than under dense spacing. The fitness also varies at different ratios of resistant to susceptible individuals in competition. Only the data from a 1:1 mixture are given in Table 1. The original data show that the resistant biotypes are even less fit than the 1:1 mixture when they are in a lower proportion in the populations. The data suggest that fitness should be measured at various densities and ratios of sensitive to resistant individuals, especially those that more closely approximate the initial low frequencies of resistant individuals in the field.

*c) Environment and fitness.* A lowered optimal temperature for growth and photosynthesis for resistant biotypes is one of the common (12) but not universal (51) pleiotropic effects found with triazine resistance. Interpretation of these findings can be complicated. The earlier germination and "head start" can be highly advantageous under many greenhouse conditions; but in the field, it can be devastating. A late frost will decimate the earlier germinating resistant population and leave the later germinating susceptible population. This demonstrates why fitness must be measured under field conditions to indicate what happens in the field.

*d) Changing seedbank dynamics.* Repeated and strong selection for resistant weeds under monoculture easily can abolish the "spaced out" germination typical of weeds during the season and over many seasons. This higher immediate germination of resistant individuals versus the susceptible in many fitness tests (e.g., 36) may give real but misleading results that do not approximate fitness properties under field conditions.

*e) Narrow genetic base.* Isozyme electrophoresis studies of resistant populations always have shown that resistant biotypes at least initially possess a much narrower genetic base than adjacent susceptible populations (10, 11, 14, 53). This is due to the "founder effect" of mutants in diverse populations measured soon after evolution. In genetic evolutionary terms, this suggests that under certain narrow conditions the resistant populations may be more fit than the wild type; but under broad and varying environmental conditions they will be less fit.

In many cases, this might mean that the fitness will increase slowly due to "nuclear compensation" from repeated crossing with the wild type susceptible population. The fundamental biochemical lesion caused by triazine resistance probably will prevent fitness from improving in that case. One can increase the yield of triazine-resistant species by intercrossing. Still the reciprocal intercrosses with the sensitive biotype as female parent always outyielded the resistant offspring in such crosses (4). The effect of interbreeding on increasing fitness will have to be checked with other types of herbicide resistance.

**The importance of negative cross resistance.** Resistant pests often are controlled more effectively than the wild type by a variety of agents. This phenomenon, known as "negative cross resistance," has been reported with antibiotic-resistant bacteria in medicine and in fungicide-resistant pathogens and insecticide-resistant arthropods (16).

Similar data can be found for weeds (Table 2), which indicates that the phenomenon should be explored as a part of resistance management procedures. Triazine-resistant individuals were often less fit under the agronomic procedures used during the "off" years when triazines were not used and procedures with negative cross resistance were used. We have no accurate counts of the decay of resistance in populations when triazine usage was stopped due to high resistance levels.

Negative cross resistance has been found with many weed control and biological factors:

a) Standard mechanical cultivations of mixed resistant and sensitive common groundsel populations reduced the resistant individuals more than the susceptible ones (54).

b) The differential lack of fitness often can be due to other biotic factors. It was found that triazine-resistant rutabagas (*Brassica napobrassica* L.), which nominally produced yields as high as the near iso-nuclear susceptible biotype, were totally and selectively decimated by a viral infection[4]. Triazine-resistant smooth pigweed was selectively eaten by beetle larvae, and triazine-resistant common lambsquarters was more susceptible to fungal disease than the wild types[5].

c) Many herbicides are more toxic to resistant individuals than to susceptible ones (Table 2). Table 2 only contains data for negative cross resistances, but these are not the preponderant cases and are not universal. Still, they can be elucidated and incorporated into strategies for managing resistant weeds, both before and after populations become preponderantly resistant.

The negative cross resistances in atrazine-resistant weeds include herbicides that act at or near the same

[4]Souza-Machado, V. 1987. Personal communication. Univ. Guelph, Guelph, Ont., Canada.

[5]Ritter, R. 1988. Personal communication. Univ. MD., College Park, MD.

*Table 2.* Negative cross resistance of herbicide-resistant biotypes.

| Primary resistance Species[a] | Negative cross resistance[b] | Parameter measured[c] | $I_{50}$[d] R/S | Ref. |
|---|---|---|---|---|
| Triazines | | | | |
| Redroot pigweed | Dinoseb | FW | 0.27 | 13 |
| | Fluometuron | Thylakoids | 0.22 | 39 |
| | DNOC | Thylakoids | 0.5 | 39 |
| Common lambsquarters | Dinoseb | FW | 0.27 | 13 |
| Rapeseed | Dinoseb | FW | 0.66 | 13 |
| Common groundsel | Dinoseb | FW | 0.21 | 13 |
| Horseweed | DNOC | Thylakoids | 0.1 | 33 |
| Kochia | 2,4-D | FW | d | 44 |
| American willowherb | Oxyfluorfen | FW | d | 8 |
| | Paraquat | FW | d | 8 |
| | Pyridate | FW | d | 8 |
| | Chlorpropham | FW | 0.46 | 6 |
| Dinitroanilines | | | | |
| Goosegrass | Chlorpropham | | d | 50 |
| Mecoprop | | | | |
| Common chickweed | Benazolin | FW | 0.53 | 35 |
| MSMA–DSMA | | | | |
| Common cocklebur | Paraquat | FW | 0.50 | 25 |
| | Bentazon | FW | 0.65 | 25 |
| Chlorsulfuron | | | | |
| Jimsonweed | Imazaquin | FW | 0.03 | 45 |
| Paraquat | | | | |
| Horseweed | Glufosinate | PS | 0.26 | 40 |

[a]Species not defined previously: horseweed [*Conyza canadensis* (L.)Cronq. # ERICA]; kochia [*Kochia scoparia* (L.)Schrad. # KCHSC]; american willowherb (*Epilobium ciliatum* Rafin. = *E. adenocaulon* Hausskn # EPIAC); goosegrass [*Eleusine indica* (L.)Gaertn. # ELEIN]; common chickweed [*Stellaria media* (L.)Vill. # STEME]; common cocklebur (*Xanthium strumarium* L. # XANST); jimsonweed (*Datura innoxia* Mill. # DATIN).

[b]Chemicals not defined previously: dinoseb [2-(1-methylpropyl)-4,6-dinitrophenol]; fluometuron [*N,N*-dimethyl-*N'*-[3-(trifluoromethyl)phenyl)urea]; DNOC (4,6-dinitro-*o*-cresol); oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene]; pyridate [*O*-(6-chloro-3-phenyl-4-pyridazinyl)-*S*-octyl carbonothioate]; chlorpropham (1-methylethyl-3-chlorophenylcarbamate); benazolin (4-chloro-2-oxo-3(2*H*)-benzothiazole-acetic acid); bentazon [3-(1-methylethyl)-(1*H*)-(2,1,3-benzothiadiazin-4-(3*H*-one 2,2-dioxide]; imazaquin {2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid}; glufosinate [ammonium (3-amino-3-carboxypropyl)methylphosphinate]; MSMA (monosodium salt of methylarsonic acid); DMSA (disodium salt of methylarsonic acid).

[c]FW = fresh weight; PS = photosynthetic $CO_2$ fixation; thylakoids = photosystem II activity of isolated thylakoids.

[d]The $I_{50}$ is the concentration lowering the parameter measured by 50%; R = resistant; S = susceptible. Where no $I_{50}$ R/S ratio is given, there was a large degree of negative cross resistance at a single herbicidal rate.

site in photosystem II (DNOC and dinoseb) as well as herbicides acting on other photosystems (paraquat) or at totally different sites. There was negative cross resistance to other tubulin-binding herbicides in dinitroaniline-resistant goosegrass (Table 2) but not to six commercial herbicides on this weed (38). The negative cross resistance to imazaquin (Table 2) occurred in only one of 21 chlorsulfuron-resistant mutants. The other mutants had varying levels of co-resistance to imazaquin.

Resistant biotypes sometimes grow better in the presence of the herbicide than without the herbicide. For instance, Lipecki[6] found that a triazine-resistant biotype of smooth pigweed grown with 5 kg $ha^{-1}$ simazine (6-chloro-*N,N'*-diethyl-1,3,5-triazine-2,4-diamine) had double the dry weight per plant than without the herbicide. This lower resistant-biotype productivity when the herbicide is not present results in a stronger lack of competitive fitness in the "off" years.

## MODIFIED MODEL FOR ROTATIONS

The long-term effect of rotational strategies is easier to calculate by deriving new equations that better consider the rotational perturbations. We have modified Equation [1] to give a new basic equation

$$H_{p,q} = [1 + \delta\,(\alpha f_{on} - 1]^p\,[1 - \delta(1 - f_{off})]^q \quad [2]$$

where:

$H$ is the overall enrichment factor giving the increase in resistance following a period of $p$ "on" seasons of herbicide application and $q$ "off" seasons without herbicide;

$\delta$, the fraction of seeds leaving the seedbank each year, replaces $\bar{n}$, the average residence time as the factor describing seed bank characters.

The derivation of Equation [2] and the simplifications, approximations, and assumptions involved are detailed elsewhere (24). Equation [2] can be rearranged to solve for various parameters to predict the effects of different agricultural scenarios. The $p$ "on" years and the $q$ "off" years can occur in any order during the $p + q$ year period that is under study.

There is little added effect of a small "off" fitness (e.g., $f_{off} < 0.3$), as there is a significant loss of resistant seeds during the off years due to the decimation of these seeds in the seedbank by natural causes (rotting, insects, etc.). This loss will not be replaced by the small addition that emanates from less fit resistant seeds. Note that the "off" factor is simply $f_{off}$ in the absence of a seedbank ($\delta = 1$), as the only factor affecting seed number will be seed deposition, however small. If $\delta = 1$, then a strong influence of $f_{off}$ can be expected.

[6]Lipecki, J. 1988. Personal communication. Adademii Rolniczej W. Lublinie, St.-Leszczynskiego 7, Lublin, Poland.

Various applications of this newer model are plotted in Figures 2 to 5, showing examples of how this model can be used to predict what might happen in real field situations, when different herbicide treatments are used in various rotations.

The effects of various rotations on the rate of evolution of resistance are plotted using Equation [2] to show the effects of fitness during the season when the herbicide was used and a different (constant) fitness for seasons when not used (Figure 2). There is hardly any real added delay due to rotation on resistance when the resistant individuals have near-normal fitness (Figures 2 D to F). Actually, Figure 1 and Figures 2 D to F do not differ agronomically if the lines in Figure 2 are smoothed. This high fitness may be the situation with the weeds having resistance at the level of acetolactate synthase (based on productivity, not competition experiments). Thus, in such cases, with high fitness, the model states that rotation is of little assistance in truly delaying resistance. The only delay will be for the number of generations the particular herbicide is not used. In such cases, only lowering the selection pressure will delay resistance. Obviously, the model must be validated by agro-ecological experimentation.

When there is a large fitness differential between resistant and susceptible individuals (as with triazine-resistant weeds), there will be a delaying effect due to fitness (Figures 2 A to C), and the effect is greatest when selection pressure is lowest. The plots describe the reduction of the proportion of resistant individuals in the off years. There are even some situations at low selection pressure where resistant individuals disappear in "off" years more rapidly than they are enriched for in "on" years. Thus, rotation can be advantageous. When there is negative cross resistance, the fitness differential is even greater, and the results can be considered by using the effects of a lower fitness value $f$ for this period.

Scenarios with a slow rate of overall enrichment, showing that it will take many years for resistant populations to become a major problem, are summarized in Table 3. The (log) factor of enrichments at the end of 9- and 15-yr periods are given. When this factor is compared with the initial frequency of resistance ($N_o$), it can be estimated whether resistant populations should have evolved. If $N_o$ is $10^{-20}$ (a guess for the $N_o$ of triazine resistance), fitness is 0.3 or less, and selection pressure is low, i.e., the effective kill is less than 95%, then we see that resistant populations will not evolve

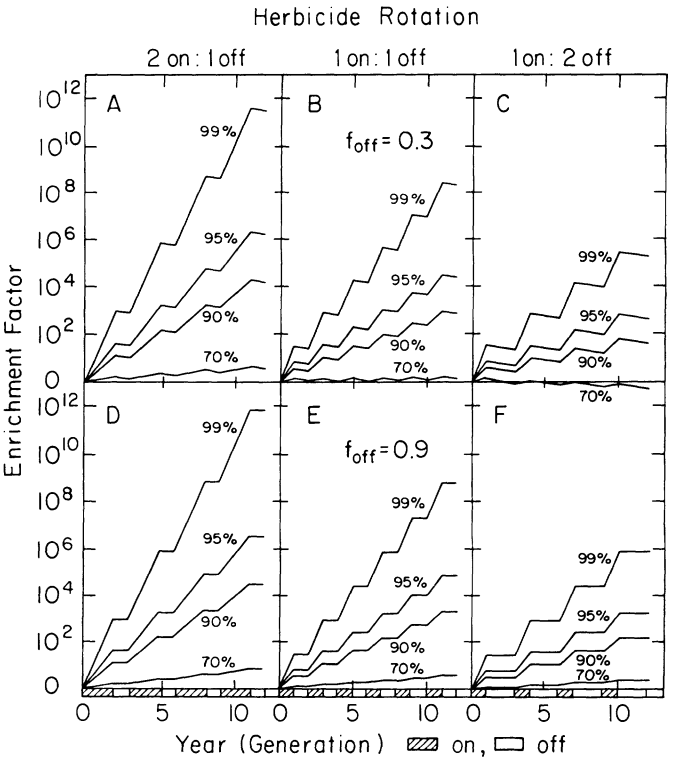


*Figure 2.* The effect of herbicide rotation on the rate of resistance enrichment. Three rotational scenarios are shown for herbicides with different selection pressures expressed as effective kill. "On" refers to the year the herbicide in question is used and "off" the year it is not used. The two fitnesses are those thought to represent triazine resistance (Table 1) (A to C; f = 0.3) and those thought to represent sulfonylurea/ALS resistance (7) (D to F; $f$ = 0.9). The $f_{on}$ = 1. The weed seeds in the seedbank are presumed to have a 2-yr residence time. Calculated from equations in (24).

under any of these scenarios. Triazine resistance would only appear in 15 yr in monoherbicide culture where the effective kill is 99% (Log $H$ = 22.3, which makes up for the resistance frequency of $10^{-20}$).

With chlorsulfuron, $N_o$ should be $10^{-6}$ to $10^{-8}$ (7, 26, 45, 48) versus the estimates of $10^{-20}$ for triazine resistance. This high initial frequency explains why chlorsulfuron resistance evolved so rapidly. It is not clear that one need actually consider whether mutations to resistance are dominant or recessive; there may be only a small frequency difference between the two types in diploid organisms (55). This is because recombination (crossing over) can increase homozygous recessive frequencies in populations considerably. The selection pressure of 2,4-D is so low that no resistant populations occurred in 35 yr of monoculture wheat (29) as would be expected from Table 3.

The important predictive uses of this model are twofold:

*Table 3.* (Log) Enrichment of resistant individuals in weed populations over 9- and 15-yr periods under different herbicide rotations[a].

| Rotation strategy | Effective kill[b] | 9-yr period | | | 15-yr period | | |
|---|---|---|---|---|---|---|---|
| | | Fitness in "off" years | | | Fitness in "off" years | | |
| | | 0.9 | 0.5 | 0.3 | 0.9 | 0.5 | 0.3 |
| | (%) | [Log 10 of enrichment factor (Log *H*)] | | | | | |
| No rotation | 50 | 1.0 | 1.0 | 1.0 | 1.7 | 1.7 | 1.7 |
| | 90 | 5.1 | 5.1 | 5.1 | 8.5 | 8.5 | 8.5 |
| | 95 | 7.4 | 7.4 | 7.4 | 12.4 | 12.4 | 12.4 |
| | 99 | 13.4 | 13.4 | 13.4 | 22.3 | 22.3 | 22.3 |
| 2 on; 1 off | 50 | 0.6 | 0.5 | 0.4 | 1.1 | 0.8 | 0.6 |
| | 90 | 3.4 | 3.2 | 3.1 | 5.6 | 5.3 | 5.2 |
| | 95 | 4.9 | 4.7 | 4.6 | 8.2 | 7.9 | 7.8 |
| | 99 | 8.9 | 8.7 | 8.6 | 14.3 | 14.5 | 14.4 |
| 1 on; 1 off | 50 | 0.5 | 0.3 | 0.2 | 0.8 | 0.4 | 0.2 |
| | 90 | 2.8 | 2.6 | 2.4 | 4.4 | 4.0 | 3.8 |
| | 95 | 4.1 | 3.8 | 3.7 | 6.5 | 6.1 | 5.9 |
| | 99 | 7.4 | 7.1 | 7.0 | 11.8 | 11.4 | 11.2 |
| 1 on; 2 off | 50 | 0.3 | –0.1 | –0.3 | 0.4 | –0.1 | –0.4 |
| | 90 | 1.6 | 1.3 | 1.1 | 2.7 | 2.1 | 1.8 |
| | 95 | 2.4 | 2.0 | 1.9 | 4.0 | 3.4 | 3.1 |
| | 99 | 4.4 | 4.0 | 3.8 | 7.3 | 6.7 | 6.4 |

[a]The table is best used to compare expected mutation frequencies for resistance and to see whether resistance would be expected with different management regimes. Examples of such frequencies would be *ca* $10^{-6}$ for a dominant monogenic trait, *ca* $10^{-12}$ for recessive monogenic mutants if unproven theory is accepted, or *ca* $10^{-7}$ if experimental data from another biological system (55) is accepted. A minus sign indicates a negative enrichment for resistance. It is presumed that the fitness of triazine-resistant weeds 0.3 to 0.5 and the fitness of ALS level sulfonylurea resistance is *ca.* 0.9. Calculated from equations in (24).

[b]The effective kill (the percent control over the whole season) of 50, 90, 95, and 99% are equivalent to $\alpha = 2, 10, 20, 100$, respectively in Equations [1] and [2]. A seed bank release of $\delta = 0.3$ (half-life in seedbank of 2 yr) and $f_{on}$ of 1.0 were assumed.

a) to design rotational and mixture scenarios to delay resistance as much as possible yet still to obtain cost-effective weed control using herbicides such as chlorsulfuron and atrazine, which are among the least expensive and most active selective herbicides for wheat and corn, respectively;

b) once resistance has occurred, to design strategies whereby herbicide usage is stopped for a number of years, until the level of resistance is below a certain proportion, and then resume limited use, during a certain proportion of the rotation cycle. Such strategies have been designed for insecticides where there already are predominantly resistant populations. These populations become diluted because of fitness and the migration of susceptible individuals into the area (16). The treatment strategies are designed so the maximum proportion of resistant individuals does not exceed a certain limit percentage.

With this newer model, a kill percentage can be calculated that will give any (within reason) desired degree of resistance enrichment after $p$ "on" years and $q$ "off" years. This was done to calculate various types of enrichment for both minimum tillage and other situations where the seed bank can be negligible. It also can be used for tillage and other seed bank situations under conditions where various acceptable levels of enrichment were allowed or to stasis where no net increase in the frequency of resistance occurs. To achieve such stasis would be nirvana. There are theoretical situations, perhaps even field situations, where stasis might be achieved. Different treatment regimes with low selection pressures giving stasis without a seedbank are shown in Figure 3. Given this information, weed control of strategies can be designed where resistant individuals will not be enriched. One cannot obtain stasis with continuous use of a herbicide under conditions giving adequate weed control. One still can ensure that the rate of enrichment is low.

The model is plotted so selection pressures that provide resistance stasis as a function of the duration of seed remaining in the seedbank are shown in Figure 4 for various fitnesses. Three possible rotation strategies are examined. Some cases can have no enrichment at all for resistance. If the effective kill by 2,4-D in wheat is only 50 to 60% due to late weed germination, then under low fitness and a 1:1 rotation there is no enrichment. Stasis can even be obtained with selection pressures above 90% if there is a 2- or more yr interval between the treatments with the herbicide. Stasis is impossible with high selection pressure herbicides in

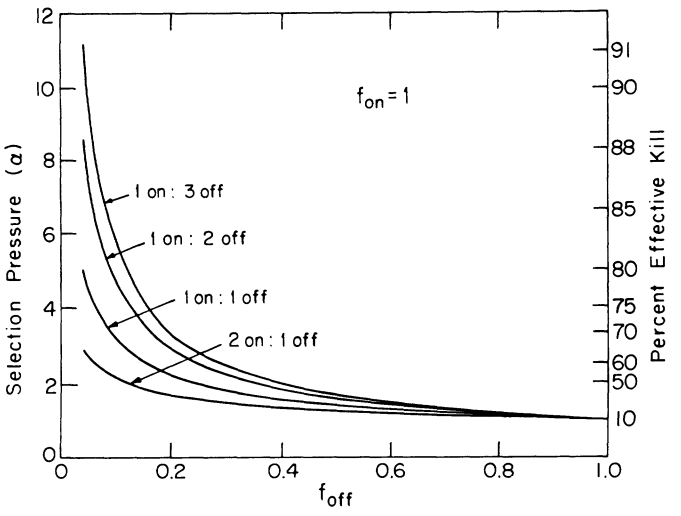


*Figure 3.* Resistance stasis for situations where there is no seedbank buffering as actually occurs in minimum-till agriculture. Values of selection pressure (α) and fitness (*f*) in "off" years that will allow no enrichment for resistance (stasis), when $f_{on}$ = 1 (24). The effective kills are based upon total lack of herbicidal effect on the resistant individuals. Calculated from equations in (24).

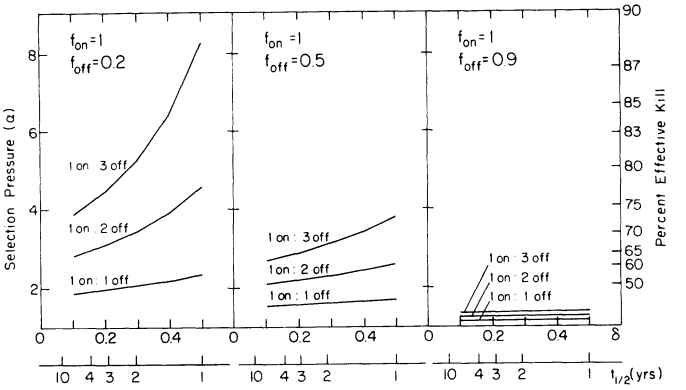


*Figure 4.* Conditions leading to a resistance stasis under various selection pressures (α), and with different rotation strategies or with different weed seed dynamics in the seedbank. The selection pressure also is shown as "effective kill" (the percent reduction in sensitive propagules over a whole season) with the assumption that the rare resistant individuals are totally unaffected by the herbicide. Here $f_{on}$ = 1 while $f_{off}$ takes a relatively low value in (A) and a higher value in (B) and (C). The $f_{off}$ of 0.2 and 0.5 in A and B approximate those found with triazine resistance, and the $f_{off}$ of 0.9 in (C) approximates the target site resistant mutants to acetolactate synthase inhibitors. The seedbank dynamics are given as δ, the fraction remaining in the soil at the end of a season, and $t_{0.5}$, the half-life of seeds in the seedbank. Calculated from equations in (24).

usual rotational sequences. Long duration of resistant seed in the seedbank is a deterrent to stasis, as resistant seeds act as a buffer for longer periods.

While stasis may be hard to achieve, a doubling of the frequency of resistant individuals every 3 yr would be acceptable. Selection pressures are depicted in Figure 5 that will just double the frequency of resistant individuals in the population in 3 yr with the "1-on: 2-off" strategy. At an intermediate duration of seed residence in the seed bank, the doubling of resistance occurs at the lowest selection pressures. This means that if the frequency of resistance is $10^{-6}$, then it will take almost 60 yr for resistant populations to predominate.

## CONCLUDING REMARKS

The model depicted from Equation [2] in Table 3 and Figures 2 to 5 describes the enrichment for resistant individuals in the population but only when they are still a minuscule proportion of the total population and not when a population actually nears resistance. As the resistant population becomes large, some of the simplifications that are valid only at lower resistant frequencies cannot be used. The complex equations from which Equation [2] was derived (24) must be used. These cast light on the new considerations that are needed for resistance management once there is a preponderance of resistant individuals. The consideration of the lower fitness in the "off" years can be refined further when actual data are available on fitness in different field situations with different species as well as more information on the dimunition of resistant populations under various agronomic and herbicide treatments.

Information on fitness also will be needed for many types of herbicide resistance to allow more accurate design of management strategies. What is clear is that all resistances so far have occurred in monoherbicide/monoculture or in equivalent situations that have the same effect. Weed species either receiving the same number of treatments, as in monoculture but over a longer period in rotational situations, or receiving mixtures that control the species have not evolved resistant populations.

Many resistant individuals are less fit (Table 1) because of the nature of the target site mutation conferring resistance or due to the high levels of gene products required to detoxify the herbicide. In other cases, resistant individuals may not be too unfit; or because of their nuclear inherited nature, many of the deleterious co-mutations may be lost. Chlorsulfuron-resistant mutants may not have lower productivity (7), but the competitive fitness of these plants has not been mea-

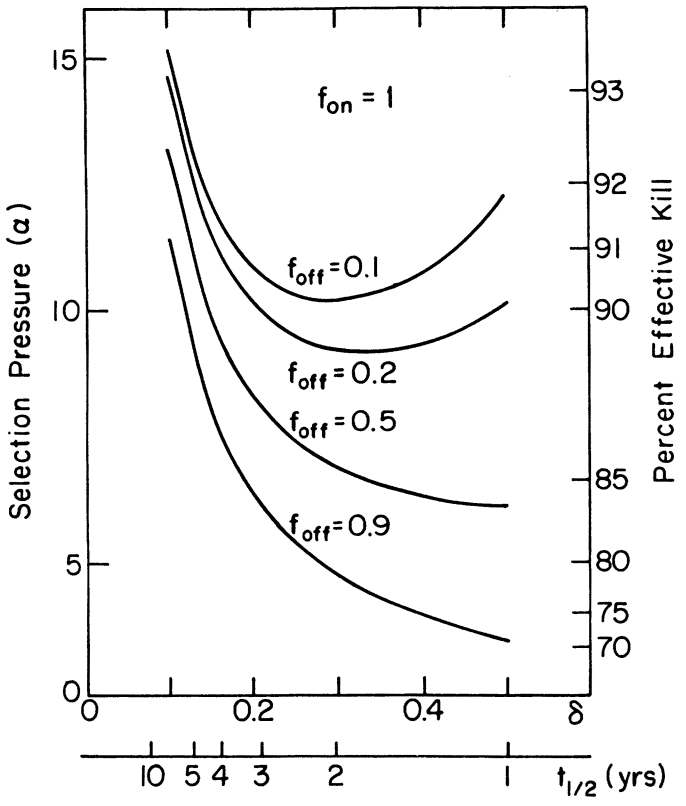


*Figure 5.* Selection pressures that cause a doubling of the proportion of resistant individuals in the population every 3 yr. The data are given for a 1 on: 2 off rotational strategy, with different fitness in the off years ($f_{on}$ = 1) and different seed bank dynamics. The seedbank dynamics are given as δ, the fraction remaining in the soil at the end of a season, and $t_{.5}$, the half-life of seeds in the seedbank. Calculated from equations in (24).

sured. There are theoretical reasons, based on the site of the mutation on the gene, to assume that these sulfonylurea-mutants need not be very unfit (46). Thus, rotating chlorsulfuron with other herbicides may not delay resistance beyond the number of "off" years, as it has with atrazine and trifluralin [2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine]. The added value of rotation will be only where the fitness is low in the off season.

Both the models and the limited field data suggest that the best tactics to prevent or to delay the appearance of resistant populations are:

a) to use herbicide treatments with the minimum selection pressure giving cost-effective weed control. Such treatments will not give near total weed control but leave behind enough susceptible seeds each year to dilute out resistant seeds;

b) to use herbicide mixtures of compounds acting at different sites of action and having different modes of degradation, preferably with herbicides having strong negative cross resistances;

c) to use rotations of herbicides having different sites of action and different modes of degradation, preferably where the weeds have negative cross resistance to the herbicides; and

d) to employ mechanical cultivations in the rotations, especially if they preferentially control unfit resistant biotypes.

These criteria are hard to meet with some monocultures, especially wheat. Wheat probably has only a single mode of degradation (19). In such situations, it is necessary either a) to rotate crops to allow herbicide rotation; b) to rotate with herbicides having a placement selectivity (e.g. 15) that is not related to herbicide metabolism in wheat; (c) to find synergists that preferentially inhibit herbicide degrading enzymes in the weeds of wheat (20).

Those high-selection-pressure herbicides having resistant mutants that are fit will be problems. The only alternative is to replace these with less persistent herbicides of the same group having less selection pressure and thus partially offsetting the lack of fitness.

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