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An Assessment, Using a Modelling Approach, of Inbreeding as a Possible Cause of Reduced Competitiveness in Triazine-Resistant Weeds.

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Abstract

1. It is widely assumed that organisms which evolve resistance to a triazine suffer from a fitness deficit in the absence of that herbicide. Arguments for this view are examined, and the published evidence is discussed.
2. A simple model is developed to examine the genetics of resistance in triazine-resistant plants, based on the assumption that there is a founder effect operating. The model examines the hypothesis that the extent to which plants in the sprayed population are related will increase rapidly under continual selection, even when there is a significant input of genes from non-selected populations.
3. The possible consequences of the above hypothesis on the validity of competition experiments are discussed.

Introduction

Triazine resistance first began to be recognized as a problem in 1969, when efforts to control *Senecio vulgaris* in a Washington nursery with the triazine herbicide simazine proved ineffectual (Ryan, 1970). Since then, resistance to triazines has been found in some 57 plant species (LeBaron 1991). Weeds are becoming resistant to an ever greater range of herbicides. In many cases a single weed biotype has been found to show resistance to a range of compounds (e.g. Moss, 1987; Pölös and Mikulás, 1987; Solymosi and Lehoczki, 1989).

The agrochemical industry acknowledges herbicide resistance in general as a cause for concern, but considers it to be a development which can be managed by the informed use of herbicide products (Dr D.J. Cole, *pers com*).

The evolution of resistance to any pesticide is widely believed to carry with it some form of metabolic cost. The argument underlying this assumption was put forward by Crow (1957) when discussing insecticide resistance:

"Since the genes causing...resistance were at low frequency in the population before the insecticide began to be applied, it must ordinarily be true that they are to some extent disadvantageous; otherwise they would have been common."

This argument has been used frequently since Crow's paper, and has led to the conclusion that, in the absence of the pesticide to which resistance has evolved, those pests with resistance will eventually die out. If this is indeed the case then it must follow that resistant mutants frequently appear in untreated populations and then become extinct; only surviving after treatment with the appropriate herbicide has begun.

A more likely hypothesis is that resistant mutants form a small but stable proportion of all populations; with the resistance genes conferring neither a selective advantage

nor disadvantage under normal circumstances. Supporting evidence for this view comes from a recent screening exercise designed to discover chlorsulfuron resistance in a previously unexposed *Lolium perenne* population (Mackenzie *et al.*, 1993). Resistant phenotypes were found in frequencies ranging from 5 to 250 plants per million.

Clearly the view that resistance automatically confers a penalty is over-simplistic; and indeed, there are recorded examples of triazine-resistant weeds which were found to be as fit or fitter than the susceptible plants with which they were grown in competition (Warwick and Black, 1981, Rubin *et al.*, 1985).

Where fungicide resistant organisms have been found to be fitter than wild-type fungi, it has been postulated that the resistant population acquired other characteristics, apart from resistance, which led to an overall increase in fitness (M.J. Jeger, *pers. com.*). This might also be the case with weeds. Madden (1988), for example, found evidence of faster germination in triazine-resistant *Senecio vulgaris*.

However, such an hypothesis implies that the selection pressures which lead to this greater fitness are coincident with the selection for resistance. Such an hypothesis would be valid only in a few, specific circumstances; and easily the greater part of the evidence suggests that triazine-resistant weeds are competitively inferior (e.g., Conard and Radosevich 1979, Holt, 1988, Madden 1988).

Indeed, the view that resistance in general leads to a fitness deficit is so widely accepted that models predicting the increase in levels of resistance often incorporate the idea of a fitness deficit (Gressel and Segal 1986). Even Maxwell *et al.* (1990) whose model does not assume that resistant plants will always be less fit than susceptible plants assigns a lower fertility parameter to resistant plants than to susceptible ones.

Because most triazine resistance is due to a single base change in chloroplast DNA, the fitness deficit has been assumed to manifest itself as a reduction in photosynthetic efficiency. Arntzen *et al.* (1979) and several later researchers found evidence for this in the fluorescence transients of triazine resistant plants. The transients they published indicated a slightly longer delay in the initiation of photosynthesis in a triazine resistant leaf than in a susceptible one. However, they gave no indication of the degree of variation in transients, or whether the differences were significant. A similar criticism applies to other studies which follow Arntzen *et al.* (Madden 1988). Madden compared fluorescence transients of several species, and found no statistically significant differences to support the accepted view of elevated fluorescence levels in resistant plants. There was, however, considerable variation.

The purpose of this paper is to explore the idea that where there is a perceived fitness penalty in triazine-resistant plants it may be due, at least in part, to inbreeding depression. Inbreeding depression is known to increase the frequency of homozygotes and decrease that of heterozygotes. Many experiments have shown that the net effect of this is to reduce the overall fitness of the population (Hedrick, 1985).

Reduction in Fitness Due to Inbreeding

Where herbicides are being applied, there is powerful selection in favour of resistant plants. If the selection is strong enough, numbers of resistant weeds will quickly increase, until they form a major part of the population. On the assumption that resistance was rare prior to treatment with the herbicide, the growing proportion of resistant weeds in the population will be descended from a small number of resistant progenitors. In the case of triazine resistance at the chloroplast level, this is particularly true. Because the resistance gene is carried on the chloroplast, it is rarely disseminated in pollen (Souza Machoda *et al.*, 1978). This feature of maternal inheritance makes the genetics of triazine resistance relatively easy to model, removing, as it does, the need for any assumptions about dominance, and so making it unnecessary to include heterozygotes in the model.

Genetics

The genetics of triazine resistance are summarized in the table below:

	P_{n+1}(ovum)	Q_{n+1}(ovum)
P_{n+1}(pollen)	$p_n^2(R)$	$p_n \cdot q_n(S)$
Q_{n+1}(pollen)	$p_n \cdot q_n(R)$	$q_n^2(S)$
	$R_{n+1} = p_n^2 + p_n \cdot q_n$	$S_{n+1} = q_n^2 + p_n \cdot q_n + q_m$

where (R) and (S) indicate which of the progeny from the cross are resistant and which susceptible. R_{n+1} and S_{n+1} give the proportion, relative to generation n , of, respectively, resistant and susceptible progeny.

In the above table:

p_n = frequency of resistant plants at generation n

(i.e. R / total gametes)

q_n = frequency of susceptible plants at generation n .

(i.e. S / total gametes)

sv = % of susceptible plants which survive spraying.

(All resistant plants are assumed to survive).

qm = genes entering the field in pollen and dispersed seeds. (These are assumed to be susceptible) .

From the above, difference equations for p_{n+1} and q_{n+1} can be derived, as follows:

$$p_{n+1} = \frac{p_n^2 + p_n \cdot q_n}{p_n^2 + (1 + sv)p_n \cdot q_n + sv \cdot q_n^2 + qm} = \frac{p_n}{p_n + sv(1 - p_n) + qm}$$

$$q_{n+1} = 1 - p_{n+1}.$$

These frequencies eventually tend towards a stable equilibrium where:

$$p_\infty = 1 - \frac{qm}{1 - sv}, \quad q_\infty = \frac{qm}{1 - sv}$$

These values are asymptotic and hence are never actually reached

Relatedness

If it is assumed that genes entering the field account for only a small proportion of the gene pool, it is likely that the plants in the population will be related. to each other in some degree. This is explored below.

In the following section, the term 'degree of relatedness' refers to the proportion of nuclear DNA shared by different organisms in the population.

The proportion of resistant plants in the field is p , and that of susceptible plants is q .

If it assumed that:

- a) pollen is available from both resistant and susceptible plants; and
- b) the amount of pollen of each type is proportional to the availability of its source (i.e. $(p \times 100)\%$ of the pollen is from resistant plants);

then the probability of each option available to a resistant plant are as follows:

- 1) Probability of a resistant plant fertilizing a resistant plant = p^2
- 2) Probability of a resistant plant fertilizing a susceptible plant = pq
- 3) Probability of a susceptible plant fertilizing a resistant plant = pq .

If plants have genes from a common ancestor, their progeny will also share genes with that ancestor. If one parent has $x\%$ of its genes from the common ancestor, and the second parent has $y\%$, the progeny will, on average, be expected to have $[(x+y)/2]\%$. So for example, if a parent is crossed with one of its offspring, the progeny can be expected to have $[(100+50)/2]\%$ of its genes in common with that parent. This being the case, if it is assumed that all resistant plants are descended from a single resistant mutant, their relationship to that progenitor can be estimated from the table given earlier. Resistant plants with a degree of relatedness of r_n will, if crossed with similar plants, produce progeny with a similar degree of relatedness. If crossed with susceptible plants, the degree of relatedness of the progeny will be the mean of the resistant and susceptible parents. This is given by the following equation:

$$r_{n+1} = \frac{r_n \cdot p_n^2 + h r_n \cdot p_n \cdot q_n}{p_n^2 + p_n \cdot q_n} = h_n r_n (p_n + 1)$$

where r_n is the degree of relatedness between the n th generation and the original resistant mutant. h_n is the mean degree of relatedness to r_0 of the gametes in a resistant \times susceptible cross and is equal to

$$\sum_i^N \left[(R_i + S_i) / 2n \right],$$

where N is the number of susceptible \times resistant crosses in the population, R_i is the degree of relatedness to r_0 of the resistant maternal parent in the i^{th} cross, and S_i is the degree of relatedness to r_0 of the susceptible parent in the i^{th} cross.

Even with experimental data it would be hard to assign an accurate value to h . However, it can be stated with certainty that it would have a minimum value of 0.5. This is equivalent to the assumption that susceptible plants are totally unrelated to resistant plants.

Model Behaviour

Levels of Resistance in the Weed Population

The model was run with resistance at an initial level equivalent to one resistant plant in a population of 100 000.

The effect on p_n of varying sv and qm is shown in Figs 1 and 2.

Fig 2 shows the importance of selection (sv) and the relatively low impact of immigration (qm) on the rate of resistance increase within the weed population. The rate of immigration, however, proves to be the most important factor in determining equilibrium values. If qm is sufficiently high, resistance levels are limited by the constant influx of susceptible genes (Fig 1).

In Fig 2, the equilibrium values for $qm = 10\%$, $sv > 0.85$ are less than or equal to 0, hence the shortened curve. In biological terms, at these levels of migration and selection, the level of resistance never builds up in the population.

Degrees of Relatedness

The model was run with the assumption that h is 0.5, i.e. that susceptible plants are totally unrelated to resistant plants. It is therefore only to be expected that r_n should decline rapidly. Consequently, rather than considering the relationship between resistant plants of the n th generation and the original resistant plant, it is more useful to look at the relationship between consecutive generations, i.e., r_{n+1} / r_n .

Fig 3 illustrates the extent to which the resistant plants are related at the 90% equilibrium level.

The fluctuations in the curves are an artefact caused by the use of a discontinuous function. The important things to note are the trends. Except in cases where the equilibrium value is low, r values are all around 0.75 or above; a value considerably higher than the 0.5 which would normally be expected between parents and progeny in a large, panmictic population. These figures are almost certainly inaccurate, due to the fact that h_n was approximated throughout using the lower bound of 0.5. The inaccuracies however, are such that they would tend to underestimate the level of relatedness.

Discussion

Fig 3 shows a tendency towards an inbred resistant population even where there is a high proportion of incoming genes. This is despite the fact that the value of h used was unrealistically low, causing the model to under-estimate systematically the level of relatedness between resistant weeds. Experimental evidence of such a founder effect has been reported. Madden (1988), Zanin and Lucchin (1990), Darmency and Gasquez (1990) and Warwick and Black (1993) have all found far greater homogeneity amongst resistant weeds than amongst susceptible weeds and have attributed it to founder effect. There is also evidence from crossing experiments. Stowe and Holt (1988) used a range of indicators to show reduced fitness in triazine-resistant *Senecio vulgaris*. When crossed with susceptible plants, the resistant F1 hybrid was found to be considerably fitter than its resistant parent. It was still less fit than the susceptible progeny from the cross, suggesting that there may be some physiological basis to reduced competitiveness, as is usually supposed; but the findings certainly provide support for the hypothesis that inbreeding is having an adverse effect.

Many experiments in which the relative competitiveness of triazine-resistant and susceptible plants have been compared have used plants from a single location. If those plants had undergone years of selection, their gene pool could have been drastically reduced. This may have been quite sufficient to account for any observed fitness deficit.

There is clearly a need for experimental work to assess the effect of pesticide selection on genetic variability within a resistant pest; and to determine the effect of any reduced variability on fitness. If this proves to be significant it has serious implications for the management of resistant weeds. In the past the view has often been expressed that, by rotating herbicides or by leaving an area unsprayed, the resistant population will eventually die out due to its supposedly reduced competitiveness. If this is merely an artefact caused by inbreeding, then resistant weeds will always form a significant part of the population.

In developed countries, where a wide range of herbicides is available for most purposes, this is not yet a problem. However, in developing countries, such as Costa Rica, where propanil-resistant *Echinochloa colonum* is becoming common, there is less choice.

Even in developed countries, resistance could become a problem, particularly given the increasing reliance on modern, highly effective chemicals which appear to select strongly for resistance. Furthermore, with more and more agrochemical companies attempting to engineer herbicide-resistant crop plants, the routine use of single herbicides over a long period of time is likely to grow, increasing the spread of herbicide resistance still further.

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Figure Legend

Fig 1: Levels of resistance at equilibrium ϕ_e for different levels of selection.
($p_0 = 0.001\%$ of the weed population)

Fig 2: The number of generations required before 90% of the resistance equilibrium level is reached.
($p_0 = 0.001\%$ of the weed population)

Fig 3: The minimum level of relatedness between generations of herbicide resistant weeds at 90% of equilibrium. (h is taken at the lower bound of 0.5)
($p_0 = 0.001\%$ of the weed population)



