Negative Cross Resistance; a Possible Key to Atrazine Resistance Management: A Call for Whole Plant Data

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Z. Naturforsch. **45c,** 470–473 (1990); received October 23, 1989

Negative Cross Resistance, Atrazine Resistance, Herbicide Resistance, Resistance Management, Photosystem II Herbicides

Many photosystem II inhibiting herbicides still inhibit this process in triazine-resistant plants; *i.e.* they have no cross resistance with atrazine. Five- to twenty-fold lower concentrations of phenolic type herbicides and 5-fold less of the active ingredient of pyridate and half as much ioxynil are required to inhibit thylakoid PS II in atrazine-resistant biotypes than in sensitive biotypes; *i.e.*, they even show "negative cross resistance".

Negative cross resistance may be the major reason that atrazine resistance did not evolve where herbicide mixtures were used, when the mixed herbicide (usually a non-PS II inhibiting acetanilide) also controlled triazine-sensitive weeds.

Mathematical modeling in principle allows quantification of the very low field levels of herbicides possessing negative cross resistance that could be mixed with atrazine that would stop or delay the evolution of resistant populations without affecting the maize crop. There are few available actual dose response curves of atrazine-resistant *vs.* susceptible weeds at the whole plant level for herbicides exerting negative cross resistance. Thus, "real situation" modeling cannot be done. Data acquisition is called for so that the model can be extrapolated from the thylakoid to the field.

Introduction

Populations of weeds resistant to atrazine have only evolved where monoculture with atrazine was continuously used for 6–10 years. There are essentially no reports of evolution of resistance where atrazine was rotated or where atrazine was mixed with other herbicides affecting the same species. Totals of well above 10 applications of atrazine were used in rotational systems in vast areas, which should have made up for the seasons when atrazine was not used. No resistance evolved [1]. Similarly, a sufficient number of seasons with mixed treatments have passed to make up for the lower frequency of double mutants that would be co-resistant to atrazine and the mixed herbicide, to theoretically allow for resistant populations to evolve [2]. No triazine-resistant populations have evolved where such mixtures were used [1].

We propose that resistance may have been delayed or suppressed because of the ability of some of the herbicides mixed with atrazine or used in rotation with atrazine to have a more severe effect on atrazine-resistant biotypes than on the susceptible biotypes, *i.e.* there is "negative cross resistance" to these herbicides.

Reports of negative cross resistance with atrazine are summarized in Table I. Herbicides known to inhibit PS II as well as an array of herbicides with other modes of action (paraquat, oxyfluorfen, chlorpropham) exert negative cross resistance on atrazine-resistant biotypes. It has been suggested that the negative cross resistance of the PS II inhibitors is due to their being highly lipophilic [7]. Triazine resistance is usually pleiotropic with compositional changes in membrane lipids to more unsaturated forms [10–12], which would better dissolve the more lipophilic herbicides. Still, this may not fully account for the large degree of negative cross resistance. The general lack of fitness found in weed biotypes that evolved resistance also delays the appearance of resistance [2, 13], and triazine-resistant biotypes may be less fit than the wild types to any herbicide stress.

Negative cross resistance is not unique to atrazine-resistant weed biotypes. It is a well known phenomenon with insecticides and fungicides and their resistant pests. It has also been documented with other herbicides (Table II).

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/90/0500–0470 $ 01.30/0

Table I. Negative cross resistance of triazine-resistant biotypes.

Species	Negative cross resistance	Parameter measured	Rate giving 50% inhibition (R/S)	Refs.
Amaranthus retroflexus	dinoseb	fresh weight	.27	[3]
	fluometuron	thylakoids	.22	[4]
	DNOC	thylakoids	.5	[4]
Chenopodium album	dinoseb	thylakoids	.27	[3]
Brassica napus	dinoseb	thylakoids	.66	[3]
Senecio vulgaris	dinoseb	thylakoids	.21	[3]
Conyza canadensis	DNOC	thylakoids	.21	[5]
Epilobium ciliatum	chlorpropham	fresh weight	.46	[6]
Brassica napus	dinoterb	thylakoids	.07	[7]
	dinoseb	thylakoids	.12	[8]
	medinoterb	thylakoids	.20	[7]
	pyridate (active metabolite)[a]	thylakoids	.21	[7]
	ioxynil	thylakoids	.45	[7]
	bromoxynil	thylakoids	.71	[7]
Kochia scoparia	2,4-D	fresh weight	R inhibited	[8]
Epilobium ciliatum	oxyfluorfen	fresh weight	more than S	[9]
	paraquat	fresh weight	at a	[9]
	pyridate	fresh weight	single rate	[9]

[a] Pyridate is metabolically activated to CL 9673, which was used directly with isolated thylakoids.

Table II. Negative cross resistance of resistant biotypes (non-triazine).

Primary resistance Species	Negative cross resistance	Parameter measured	Rate giving 50% inhibition (R/S)	Refs.
Dinitroaniline				
Eleucine indica	chlorpropham		Single rate	[14]
Mecoprop				
Stellaria media	benazolin	FW	.53	[15]
MSMA-DSMA				
Xanthium pensylvanicum	paraquat		.50	[16]
	bentazon		.65	[16]
Chlorsulfuron				
Datura innoxia[a]	imazaquin	FW	0.03	[7]
Paraquat				
Conyza canadensis	glufosinate	PS	.26	[18]

[a] Only in one mutant of many. The other chlorsulfuron-resistant mutants had positive cross resistance to imazaquin.

The Model

Mathematical modelling describes the rapid evolution of triazine resistance in weed populations in a monoculture situation (Fig. 1, thin line). The relative rate of evolution is described in a (simplified) equation [13]:

$$N_n = N_o \left[1 + \frac{f(\alpha_r/\alpha_s) - 1}{\bar{n}} \right]^n$$

which states that:

N_n, the proportion of atrazine resistant individuals in a population of a given species after (n) years of treatment equals:

N_o – the initial frequency of resistant individuals in the population, times a factor including

f – the overall fitness of the resistant individuals compared to the wild type. In the vast majority of cases the fitness of atrazine-resistant biotypes ranges from 0.1 to a 0.5, with exceptions.

$\bar{n}$ – the average life time of seeds in the soil seed bank and;

The selection pressure defined by two factors:

α_r – the proportion of resistants remaining after herbicide treatment, divided by

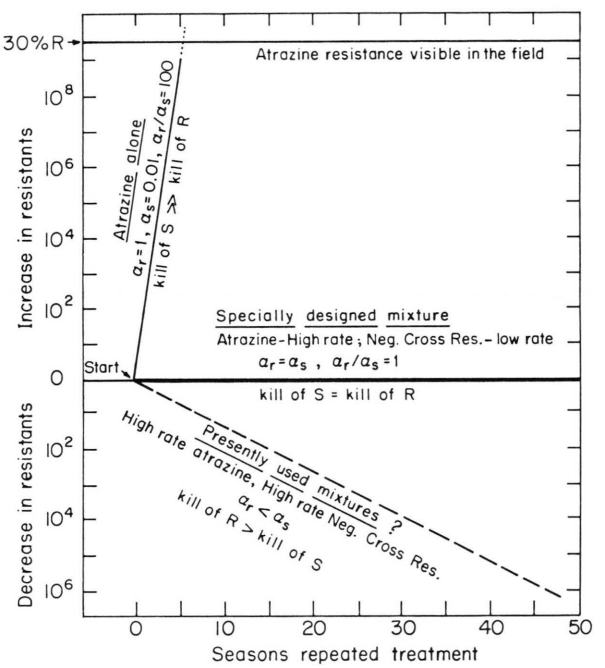

Fig. 1. Modeling the effect of negative cross resistance on the rate of evolution of atrazine resistance. *Thin line* – the rate of evolution of atrazine resistance when no other herbicide is used. It is assumed that atrazine has no effect on resistant biotype individuals and 99% effective kill (season long depression of seed yield) on susceptible biotype individuals. *Dashed line:* The effect of herbicides having strong negative cross resistance when mixed with atrazine at normal use rates. These herbicides exerting negative cross-resistance control resistant biotypes more effectively than atrazine controls the susceptible biotype. *Thick line:* The effect of specially balanced mixtures of atrazine (normal rate) and a herbicide exerting negative cross resistance (used at a low rate). The rates are balanced such that atrazine controls the susceptible biotype to the same extent that the mixed herbicide control the resistant biotype. It is assumed (for the sake of simplicity) that the mixed herbicide is ineffective on the susceptible biotype at such a low rate.

α_s – the proportion of resistant individuals remaining.

In the case shown in Fig. 1, 99% effective control of susceptibles and no control of resistants, $\alpha_r = 1$ and $\alpha_s = 0.01$ giving a selection pressure of $1 \div 0.01 = 100$.

The same model can be used to describe what may have happened if the presently used mixtures affect the atrazine-resistant biotypes to a far greater extent than the sensitive biotypes. The selection pressure for resistance is negative, as $\alpha_r < \alpha_s$, as shown in Fig. 1 (dashed line). This may be the case with pyridate and dicamba, the main herbicides used (in mixture with atrazine) in Europe to control triazine-resistant weed populations, after they evolve. Other herbicides such as alachlor and metolachlor, which normally control *Amaranthus* and *Chenopodium* spp., may exert negative cross resistance when mixed with atrazine, but no evidence is available. These chloroacetanilides are the herbicides mostly widely mixed with preemergence applied atrazine, by virtue of their excellent grass weed control.

It is not known whether the 2–10-fold lower rates of herbicide required to inhibit thylakoids from resistant *vs.* susceptible biotypes (Table I) will carry over to the field situation. If they do, one could use far lower rates of these herbicides to achieve the identical level of weed control of the resistant biotype as atrazine gives with susceptible biotype. If equal resistant/susceptible weed control rates can thus be achieved with a balanced herbicide mixture, there will be no selection pressure for atrazine resistance and no enrichment of resistance in the population (Fig. 1, thick line). A balanced herbicide mixture could be used prophylactically, *i.e.* before resistant populations predominate or just after resistance occurs. Such lower rates of herbicides exerting negative cross resistance will be much more cost effective than full doses. The low rates of herbicides exerting negative cross resistance may also allow the use of herbicides that are nominally phytotoxic to maize at their normal use rates, but without crop phytotoxicity at the lower rates.

The use of herbicides exerting negative cross resistance is not limited to use in mixtures with triazines. They can be used in rotational years. For example: if the veracity of the data in Table I vis a vis pyridate and/or ioxynil is borne out in field condi-

tions, and if 2,4-D exerts no cross resistance (negative or positive) to triazine-resistant weeds, then 2,4-D mixtures with these herbicides in a wheat rotation could selectively diminish the population of triazine-resistant weeds. Many such scenarios can be developed, but the "ifs" must be replaced by data.

Conclusions

Data needed to elucidate the magnitude of negative cross resistance at the whole plant level are lacking. The mathematical modelling shows how such data can be used as "preventative" and "after the fact" strategies to "manage" atrazine resistance, the most widespread resistance that has appeared. The same strategies can be used when negative cross resistance is found with other herbicide resistances.

It is essential to note that even in the 'best case analysis' shown in Fig. 1, that the rate of depletion utilizing negative cross resistance is much slower than the rate of increase when triazines are used alone. If/when this negative cross resistance is used in weed control strategies for a few years after resistant populations predominate, there will still be a dangerous frequency of resistant individuals in the population. Resistant populations could quickly reappear once the use of the herbicide with negative cross resistance is stopped.

The first advent of any new resistant population of resistant weeds provides the tool for studying negative cross resistance. This in turn can be used to test resistance management strategies based on negative cross resistance to preclude further evolution of resistant populations. When resistance has not yet evolved, it would be well worthwhile to consciously select for resistant weeds by severe selection pressure, to have the needed plants for studies on negative cross resistance to provide information for prophylactic management.

Those involved with production of herbicides and those engaged in management of resistant weeds are encouraged to try to obtain the necessary whole plant data on negative cross resistance and test the strategies outlined above in field situations. In places where resistance has occurred, the depletion of resistant populations should be followed using various rates of herbicides exerting negative cross resistance to test the model.

Acknowledgements

J. G. has the Gilbert de Botton Chair of Plant Sciences and L.A.S. has the Henry and Bertha Benson Chair of Mathematics.

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