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ISOENZYMATIC VARIATIONS IN POPULATIONS OF *CHENOPODIUM ALBUM* L. RESISTANT AND SUSCEPTIBLE TO TRIAZINES

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ABSTRACT

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As part of a study of triazine resistance in weed species, the authors have collected several resistant populations of *Chenopodium album* in different areas in France. A study of intra- and inter-population variability between different susceptible and resistant populations has been made using polyacrylamide gel electrophoresis of foliar isoenzymes. The separation of esterases and malate dehydrogenase isoenzymes was carried out in a modified vertical apparatus.

Within each resistant population, all individuals have the same pattern of esterase and populations collected in the same area have the same zymogram. However, populations from different areas have distinct zymograms. These data suggest that the resistant populations originated in the region where they now occur, and that there is a high rate of self-pollination in each resistant population.

After treating a susceptible population with a low dose of atrazine, about 1% of plants survived and these have a high frequency of the esterase zymogram typical of resistant individuals. The offspring of these plants are completely resistant. The resistant and susceptible populations grew near each other so that they may have a common origin. The pattern of occurrence of resistance in relation to its inheritance and the level of inbreeding in populations is discussed.

INTRODUCTION

In France, the systematic and repeated treatment of maize monocultures with triazine herbicides at first favoured summer grasses (e.g. *Echinochloa crus galli* (L.) P.B.) which initially demonstrated a range of tolerance to triazines, but from which more and more resistant plants have been selected (Gasquez and Compoint, 1977).

However, in recent years in both Europe and North America, resistant populations have appeared in weed species which had previously been very susceptible to triazines. Biotypes of *Senecio vulgaris* L. which were resistant to atrazine and simazine were first reported by Ryan (1970) occurring in a plant nursery in Western Washington, U.S.A., after ten years repeated treat-

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ment with these herbicides. Subsequently triazine resistance has evolved in other species in various other locations in the U.S.A. and Canada, mainly associated with the use of atrazine in maize crops. For example, *Chenopodium album* L. (Bandeen and McLaren, 1976), *Amaranthus retroflexus* L. (Thompson et al., 1974), *Ambrosia artemisiifolia* L., *Brassica campestris* L. (Souza Machado et al., 1977) and *Chenopodium strictum* Roth. (Warwick et al., 1979).

In France, we have observed the successive appearance of atrazine resistant populations of *Chenopodium album* L. and *Poa annua* L. (Ducruet and Gasquez, 1978), *Solanum nigrum* L. and *Polygonum lapathifolium* L. (Gasquez and Barralis, 1978) and *Amaranthus retroflexus* L., *Chenopodium polyspermum* L. and *Polygonum persicaria* L. (Gasquez and Compoint, 1980). The mechanism of resistance to atrazine in many of the weed species previously mentioned is very different from, for example, resistance to atrazine in maize which is due to degradation of the herbicide in the plant (Hamilton and Moreland, 1962). In many weed species, the mechanism of triazine resistance involves selective binding of the herbicide by chloroplast membranes (Arntzen et al., 1979).

Chenopodium album is the species with the largest number of triazine resistant populations in France. Therefore we chose to compare populations of *Chenopodium album* from several different locations to determine whether different populations have common resistance genes or whether their genotypes are different. In order to investigate the origins and geographic spread of triazine resistance, we have attempted to determine whether resistance is exogenous (through the accidental introduction of existing resistant genotypes from other areas) or whether it is indigenous. In the latter case, it is necessary to understand how resistant populations could be selected from susceptible ones.

Electrophoresis has been used to study intra- and inter-population variability In addition to herbicide treated populations we have been able to compare the frequencies of the zymograms of a susceptible population which had never been herbicide treated but which grew in the proximity of a resistant population and also plants which were selected from the susceptible population by a low dose of atrazine.

MATERIALS AND METHODS

Seeds of eight populations of *Chenopodium album* were collected from maize fields which had been treated with atrazine and from one population growing in a garden where the chemical had not been used (Table I). In each population, seeds were collected from at least fifty plants. Resistance to atrazine was tested in a greenhouse by treating seedlings with atrazine at a rate equivalent to 1000 g ha^{-1} a.i. (active ingredient). The enzymatic analyses were carried out on plants grown in a greenhouse or in a controlled environment chamber (16 h day at 25°C, 8 h night at 20°C).

TABLE I

Origin of the populations

Location of the population			Culture	Years of treatment with atrazine
No.	Site	Department		
77-1	Suvigne	Maine et Loire	Maize	4
77-5	Le Lion d'Angers	Maine et Loire	Maize	10
77-6	Le Lion d'Angers	Maine et Loire	Garden	0
78-7	La Pommeraye	Maine et Loire	Maize	>5
78-8	St. Philbert	Loire atlantique	Maize	>5
78-9	Fay de Bretagne	Loire atlantique	Maize	>5
78-10	Chateauneuf	Eure et Loir	Maize	3
78-12	St. Prim	Isère	Maize	>5
78-13	Aire S/l'Adour	Landes	Maize	>5

Triazines inhibit reactions of electron transport coupled to photosystem II, increasing fluorescence of the leaves. The degree of fluorescence in the treated plants of population 77-6 was measured by a test based on observations of whole leaves which have absorbed the herbicide atrazine (Ducruet and Gasquez, 1978). Thus, leaves of atrazine susceptible plants show a high and constant level of fluorescence due to the inhibition of electron transport. On the other hand, leaves of resistant plants show a low level of fluorescence that indicates a regular photosynthesis.

Each population sample was composed of at least fifty plants except population 77-6. For this all ninety-one surviving plants, after treatment with atrazine, were analysed. Enzyme extraction was carried out at 2°C with 250 mg leaves ground in a mortar together with 500 μl of tris buffer 0.1M, pH 7.5, containing 1% sodium thioglycolate. Samples of 40 μl were taken out of the supernatant liquid after centrifuging for 15 min. at 15000 *g*.

Electrophoresis

The electrophoretic separation used slab polyacrylamide gels in a discontinuous system, with pulsed power. This process has been described by Gasquez and Compoint (1976).

In order to improve the refrigeration of the system and to facilitate withdrawal of the gels, we used a tank which was constructed as a single vessel in "altuglas'. In the centre was a U-shaped partition against which the cell containing the gel was placed, thus dividing the vessel into two tanks. The gel cell was cooled on both sides by the thermal inertia of the tank buffer which had been previously cooled (Fig. 1). The cell containing the gel was made of two sheets of glass (one with a notch at its upper edge) separated at both ends by a plastic band thus delimiting the width of the gel. During

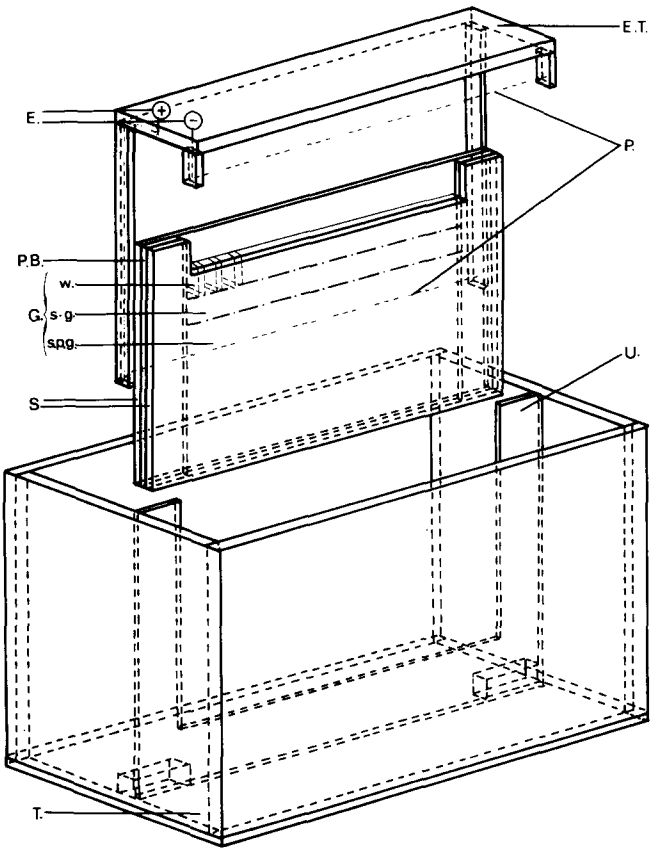

Fig. 1. General view of the apparatus, buffer tank, cell and electrode tray. T, buffer tank; U, U-shaped partition; S, sheets of glass; P.B., plastic band; G, gels (w, walls; s.g., spacer gel; s.p.g., small pore gel); E.T., electrode tray; P, platinum; E, electrodes.

preparation of the gel, the sheets of glass were held together with grease and clamps. After electrophoresis, it was necessary only to take off the plastic bands to withdraw the gel from the sheets of glass.

Several enzymes were tested (α and β esterases, peroxidases, malate dehydrogenase and acid phosphatases) and from these the α esterases and the malate dehydrogenase (MDH) were selected for comparison of the *Chenopodium album* populations. The β esterases were very similar to the α esterases; the peroxidases and the acid phosphatases, although the populations differed somewhat, were generally not variable. The staining procedures were described by Gasquez and Compoint (1977).

RESULTS

Comparison of the enzymes of resistant populations

The analysis of the plants from resistant populations shows that for each population, the zymograms are very homogeneous. Each population has exactly the same α esterase zymogram. The MDH also vary very little, some populations have only one zymogram whilst others have two (Fig. 2). The populations from the Angers area (77-1, 77-5, 78-7, 78-8, 78-9) have identical α esterases, populations from other areas show different α esterase zymograms. The populations from the Angers area are composed of only two MDH zymograms, with different frequencies (Fig. 2).

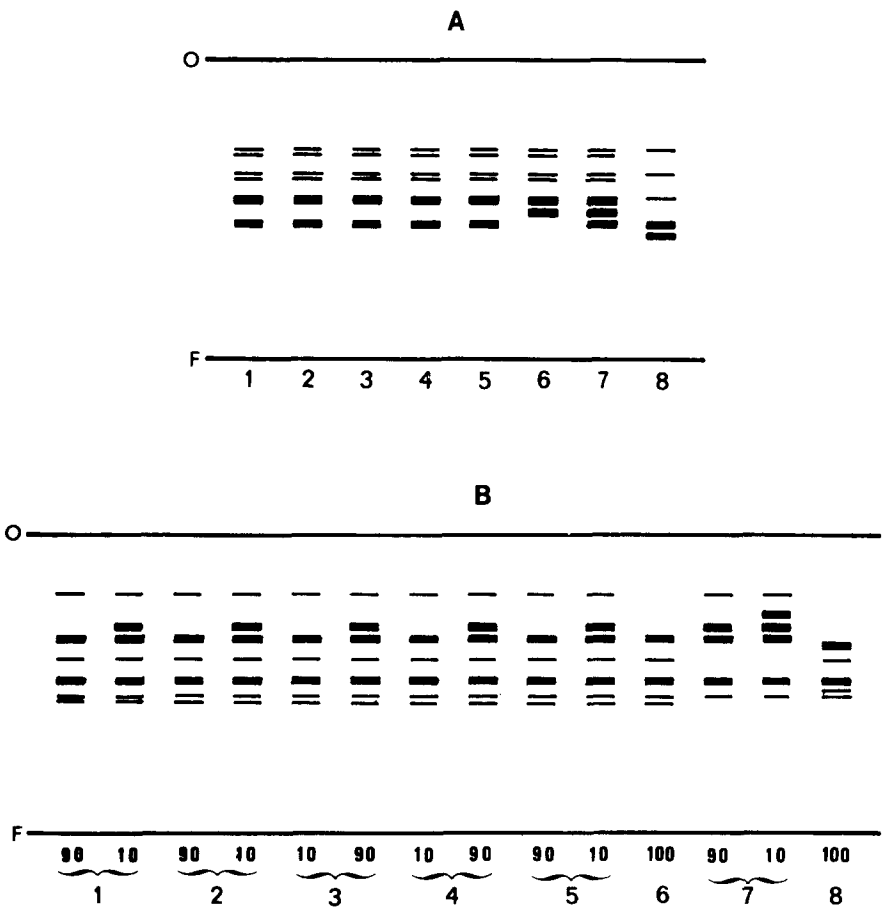

Fig. 2. Diagram of electrophoretic patterns from 8 resistant populations. A, diagram of esterase isoenzyme patterns; B, diagram of MDH isoenzyme patterns. O, origin; F, front; 1, 77-1; 2, 77-5; 3, 78-7; 4, 78-8; 5, 78-9; 6, 78-10; 7, 78-12; 8, 78-13. The numbers above the braces correspond with the frequencies of the MDH zymograms.

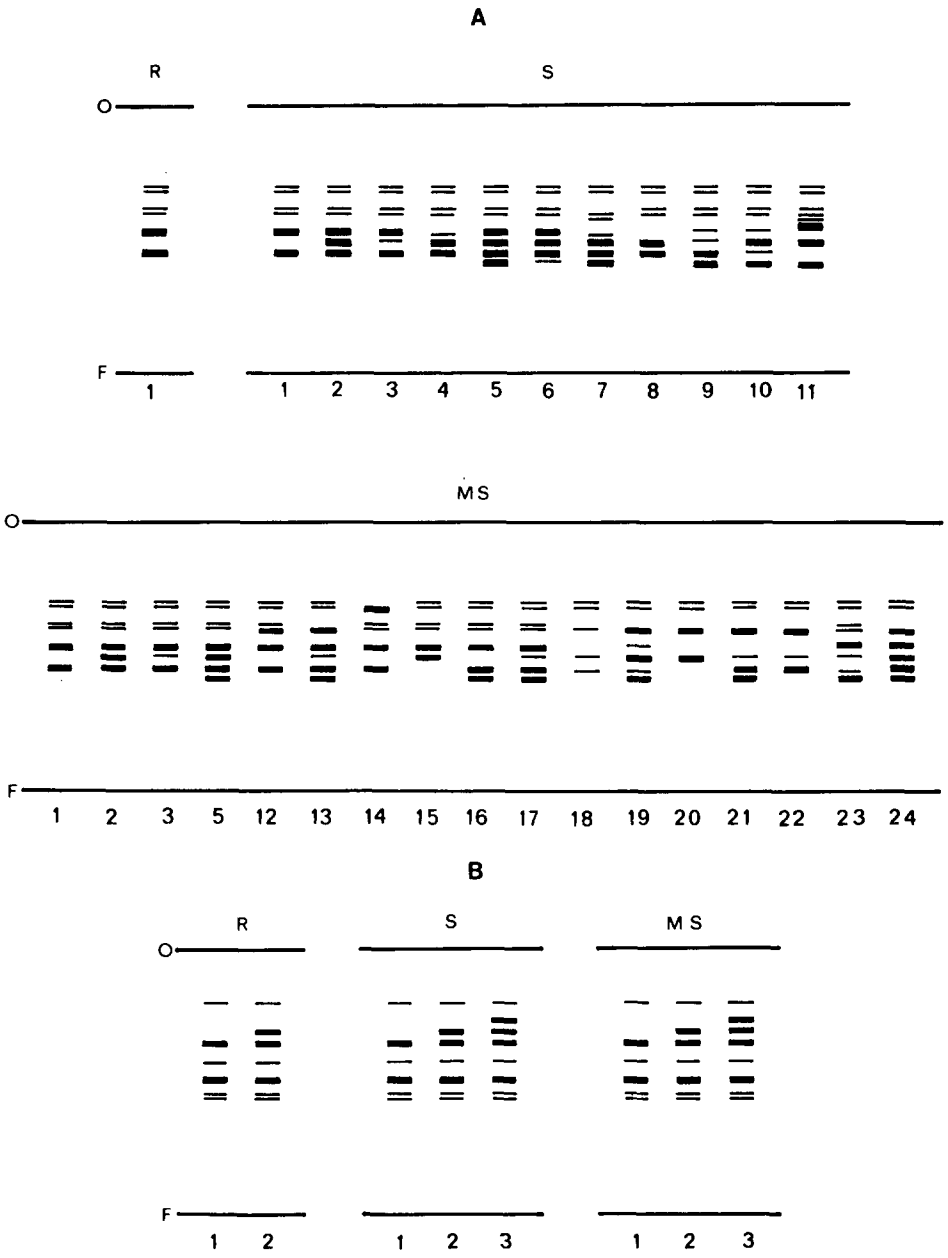

Fig. 3. Diagram of electrophoretic patterns from "R", "S" and "MS" populations. A, diagram of esterase isoenzyme patterns; B, diagram of MDH isoenzyme patterns. O, origin; F, front. The numbers correspond with the list of zymograms.

We have analysed self-pollinated offspring of several plants from population 77-5. In all cases, the α esterase zymograms are identical, only the MDH show some variability. Zymograms of the offspring are always identical to the parental zymogram. These data suggest a great genetic homogeneity of the resistant populations and parallel the relatively uniform morphology and development of plants from resistant populations (Gasquez and Barralis, 1978). However, in spite of this great genetic homogeneity these populations are certainly not completely homozygous.

Comparison of populations 77-5 and 77-6

More than 10,000 seedlings of population 77-6 were treated with atrazine (500 g ha^{-1} a.i.). Ninety-one moderately susceptible plants ("MS") were obtained which differed greatly from one another in morphology. Figure 3 shows the different zymograms occurring in resistant population 77-5 ("R"), susceptible population 77-6 ("S") and in the selected individuals from population 77-6 ("MS"). With the α esterases, it is mostly the faster moving bands which vary. Population "R" is composed only of zymogram 1. This zymogram is found in only 13% of the "S" population and in 65% of the "MS" population. In addition, there are respectively 16 and 10 other zymograms in the "MS" population and the "S" population (Table II). If we have observed less zymograms in the "S" than in the "MS", this is perhaps due to the fact that those which are specific to the "MS" are very rare. Thus a very large sample of "S" would have been necessary to observe zymograms whose frequency in the original population might be about 1/10,000.

TABLE II

Frequencies of the different esterase zymograms from "R", "MS" and "S" populations

Zymograms	Populations			Zymograms	Populations		
	"R"	"MS"	"S"		"R"	"MS"	"S"
1	100	64.8	14.3	13		3.3	
2		5.5	48.2	14		2.2	
3		9.9	12.5	15		1.1	
4			9.0	16		1.1	
5		2.2	3.6	17		1.1	
6			3.6	18		1.1	
7			1.8	19		1.1	
8			1.8	20		1.1	
9			1.8	21		1.1	
10			1.8	22		1.1	
11			1.8	23		1.1	
12		1.1		24		1.1	

With the MDH there are three zymograms in all; only two occur in the "R" population, one being of much greater frequency. The "MS" and "S" populations have all three zymograms but in the "S" population the third zymogram is the most frequent and in the "MS" is the least frequent (Table III).

TABLE III

Frequencies of the three "MDH" zymograms from the "R", "MS" and "S" populations

	1	2	3
"R"	87.5	12.5	0
"MS"	32.0	41.0	27
"S"	23.0	30.0	47

The greater diversity of the zymograms of the "MS" population indicates that there are among the population which has never been herbicide treated, resistance genes remaining at a very low level of frequency, due to a certain amount of outbreeding and gene flow between populations.

The selection pressure exerted by the herbicide is favourable for resistant individuals. However, due to competition, only the fittest will survive. This might explain the reduction in the number of zymograms in resistant populations. However, if in a given area, complete resistance is always correlated with a single zymogram, this is not necessarily due to inbreeding. Self-pollinated offspring of a few "MS" individuals are very resistant, whatever the zymogram of the parent plants may be. The fluorescence graphs of these individuals are very similar to those of resistant plants (Ducruet and Gasquez, 1978). Treatment with atrazine at a rate equivalent to 4500 g ha^{-1} in a greenhouse, was ineffective. This closely parallels the results of a selection experiment with *Senecio vulgaris* (Holliday and Putwain, 1977). If the reproductive conditions of the "MS" individuals which have experienced one cycle of selection with atrazine are favourable, then resistant individuals occur in the second year and subsequently.

CONCLUSIONS

There are several resistant populations of *Chenopodium album* originating from different areas in France. The enzymatic homogeneity of each population is considerable. Five populations from the same area (growing within a radius of 40 or 50 km) have the same esterase zymograms and very similar MDH zymograms. However, there are considerable genotype differences between populations from different areas.

The fact that we can select plants having a low level of atrazine resistance in a population (77-6) which has never been treated with the herbicide and the

biochemical heterogeneity of this population, strengthen the hypothesis that there is an indigenous resistance floating in populations which have never received a triazine herbicide treatment. The very great homogeneity of the resistant populations and the acquisition of a relatively high level of resistance from the second generation onwards suggest that there is a high percentage of inbreeding. In addition, the level of homogeneity in resistant populations may also reflect the mode of inheritance of triazine resistance. Our unpublished data concerning the offspring of resistant and susceptible plants of *Chenopodium album* which have been cross-pollinated and segregation of progenies from crosses between resistant and susceptible plants of *Solanum nigrum* indicate that triazine resistance is to a great extent transmitted by the female parent. This could account for the genetic homogeneity of resistant populations in a particular area; such populations might conceivably consist of one resistant genotype.

Inheritance of resistance to atrazine has also been investigated in Canadian populations of *Chenopodium album* (Warwick and Black, 1980) and *Brassica campestris* (Souza Machado et al., 1978). They found that there was uniparental inheritance through the female parent. Thus our conclusions concerning the genetic structure of *Chenopodium album* populations in France may be generally applicable to triazine resistant populations of other species.

Herbicide resistance is selectively favoured only by a special kind of environment. It is the recourse to maize monoculture and the repeated, systematic use of triazine herbicides which are responsible for the evolution of resistant populations. Their destruction and moreover the means to prevent their evolution are mainly dependent on crop rotation. Indeed the only selective superiority of these slow-growing and late-flowering resistant plants is due to their resistance. Pre-sowing herbicide treatments considerably favour resistant genotypes since seedlings of susceptible individuals and other species are killed and thus competition with resistant individuals is prevented. Competition would normally reduce the frequency of resistant genotypes in a population since their ecological fitness is generally less than the fitness of susceptible genotypes (Conard and Radosevich, 1979; Gasquez et al., 1980).

The properties of the populations we have investigated indicate that when highly selective herbicides are used repeatedly in crop monocultures, particularly if the herbicide has a biochemical mode of action which operates on a single metabolic pathway or at a specific site, then there will be a significant increase in the probability of evolution of resistance. This will considerably reduce the long term weed control benefits which are derived from the use of herbicides.

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