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CHROMOSOMAL ALTERATIONS INDUCED BY THE THIOCARBAMATE HERBICIDE MOLINATE (ORDRAM) IN *Vicia faba*

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ABSTRACT

Primary roots of *Vicia faba* were exposed to 25, 50, 100, 200, 300 and 400 ppm of the thiocarbamic herbicide molinate (ordram) for 4 h with recovery periods of 3, 8 and 14 h. The cells were scored for chromosome aberrations in metaphase and anaphase as chromatid and isochromatid breaks, and chromatid rings in the former and single fragments and bridges, chromosomes with inactivated centromeres, isochromosomes and alterations in the mitotic spindle in the later.

The results showed this herbicide induced aberrations when exposed to 25, 50, and 100 ppm and at 200 ppm and higher concentrations it caused cell death. Treatments of 4 h with 3 h of recovery did not produce any aberrations. This means that molinate induced a delay in the production of aberrations and when they appeared there were of chromatid type. Thus, molinate could be considered a S-dependent agent, which is in agreement with the alkylating activity of such a herbicide.

RESUMEN

Las raíces primarias de *Vicia faba* fueron expuestas a 25, 50, 100, 200, 300 and 400 ppm del herbicida tiocarbámico molinate (ordram) durante 4 horas con tiempos de recuperación de 3, 8 y 14 h. Las aberraciones fueron analizadas en células en metafase y en anafase como rompimientos cromatídicos e isocromatídicos y anillos cromatídicos en la primera y fragmentos y puentes sencillos, cromosomas con el centrómero inactivado, isocromosomas y alteraciones en el huso mitótico en la segunda.

Los resultados mostraron que este herbicida indujo aberraciones en 25, 50 y 100 ppm y en 200 ppm y a concentraciones mayores causó la muerte celular. En los tratamientos de 4 h con 3 h de recuperación no se provocaron aberraciones.

El hecho de que el molinate produzca retardo en la aparición de las aberraciones y que éstas hayan sido del tipo cromatídico, implica que tiene un comportamiento de agente S-dependiente, lo cual está de acuerdo con la actividad alquilante de dichos herbicidas.

INTRODUCTION

The thiocarbamates are an important group of herbicides used in the protection of a wide range of crops in world agriculture due to their effectiveness in controlling wild oats in cereals and crop diseases. These herbicides are volatile, although stable in acid conditions, have a relative short persistence in soils due to rapid decrease by microbial metabolism, volatization and photodegradation (Kaufman 1976) and show low toxicity in mammals (Casida *et al.* 1975).

In regard to genetic response, metabolic activation converted diallate and triallate to a mutagen in *Salmonella typhimurium* by pair-base substitution (strains TA100 and TA1535) (De Lorenzo *et al.* 1978, Sikka and Florczyk 1978) and by frameshift mutation (strain TA98) (Sandhu *et al.* 1984). Both produced positive results but in the mouse lymphoma L5178 $YTk^{+}/^{-}$ mutation assay only in the presence of metabolic activation (Sandhu *et al.* 1984). In mammalian cells in culture, diallate and triallate induced chromosomal aberrations and sister chromatid exchanges (Douglas *et al.* 1981). Both herbicides were ineffective for gene conversion, mitotic crossing-over and reverse mutation in *Saccharomyces cerevisiae* (Sandhu *et al.* 1984). Growth of tumors appeared in rats exposed to diallate (Innes *et al.* 1969, Ulland *et al.* 1973). Vernolate and butilate, another thiocarbamate herbicides, as well as diallate, produced sex-linked recessive lethal mutations in *Drosophila melanogaster* but triallate did not (Murnik 1976, Sandhu *et al.* 1984).

Molinate is a pre-emergent thiocarbamate herbicide broadly used in Mexican agriculture mainly in rice crops for the control of *Echinochloa*. In the few genetic studies carried out on this herbicide it is considered mutagenic for prokaryons (Carere and Morpurgo 1981) and positive for the induction of micronuclei in mouse bone marrow (Pintér *et al.* 1990).

Due to the lack of information on the genetical effects of molinate (alternate name, ordram) and since it is frequently used in agriculture, in this work and attempt will be made to determine its action at the citogenetic level using root tip meristems of *Vicia faba*.

MATERIALS AND METHODS

Seedlings of *Vicia faba* with a primary root length of 4 cm were exposed to 25, 50, 100, 200, 300 and 400 ppm, concentrations of molinate (S-ethyl hexahydro-1H-azepine 1 carbothioate), for 4 hours with 3, 8 and 14 h of recovery periods. Control samples were kept under the same experimental conditions, except that distilled water was used instead of the pesticide. The treatments as well as the recovery were carried out in the dark at 19°C.

The root tips after treatment were divided into two lots, one was treated with 0.05% of colchicine for 3 h and the other was fixed immediately.

The slides were stained with Feulgen reagent and made permanent by using dry ice, dehydrating with two changes of butanol and mounting in Canada balsam. Aberrations were scored in metaphase and registered as chromatid and isochromatid fragments and chromatid rings. In addition, cell analysis during anaphase were made to identify aberrations (bridges and fragments), to screen disturbances in the centromere (chromosomes with inactivated centromeres and isochromosomes) and in the mitotic spindle (multipolar anaphases). The data represent one experiment and its replicate. Slides were handled by code to avoid knowing to which group they belonged.

RESULTS AND DISCUSSION

Aberrations were observed in metaphase and anaphase (Tables I to IV) twelve hours after the begining of treatment, that means in the treated groups with 8 hours of recovery.

There were no aberrations in metaphases after 4 h of exposure and 3 h of recovery. However, with the same treatment and 8 and 14 h of recovery, chromatid and isochromatid fragments increased with the increment of the molinate concentrations; above 100 ppm necrotic tissues were produced and cell death was caused (Tables I and II).

TABLE I. ABERRATIONS (—CONTROL) INDUCED IN METAPHASE CELLS BY MOLINATE WITH 4 HOURS OF TREATMENT AND 8 HOURS OF RECOVERY

Concentrations *ppm*	*Abnormal metaphases*[a] %	*Chromatid and isochromatid fragments* %	*Chromatid rings* %	*Total aberrations* %
25	4	4	0	2.00
50	15	13	2	7.50
100	23	21	2	10.00
200	C e l l	d e a t h		

[a] 200 metaphase cells were analysed in each case.

TABLE II. ABERRATIONS (—CONTROL) INDUCED IN METAPHASE CELLS BY MOLINATE WITH 4 HOURS OF TREATMENT AND 14 HOURS OF RECOVERY

Concentrations ppm	*Abnormal metaphases** %	*Chromatid and isochromatid fragments* %	*Chromatid rings* %	*Total aberrations* %
25	2	2	0	1.00
50	8	7	1	1.50
100	32	30	2	13.50
200	C e l l	d e a t h		

[a] 200 metaphase cells were analysed in each case.

The fact that aberrations appeared after 8 and 14 h from the beginning of the treatment indicated that molinate behaved according to the statement of Kihlman (1963, 1966) as an agent with delayed effects for the appearance of aberrations. Due to this characteristic and the fact that the aberrations are of the chromatid type, this herbicide should be considered as a S-dependent agent and DNA synthesis is required for aberrations to be expressed (Kihlman *et al.* 1978). To this group belongs the alkylating agents as in the case of molinate and other carbamic pesticides.

In order to determine the effects of molinate on the centromeric region, anaphase cells were also scored looking for chromosomes with inactivated centromeres and isochromosomes (Ramanna and Natarajan 1966, Nicoloff and Gecheff 1976, Gómez-Arroyo and Villalobos-Pietrini 1983, Gómez-Arroyo *et al.* 1985, 1986) and multipolar anaphases produced by spindle disturbances (Gómez-Arroyo *et al.* 1986). Tables III and IV show a similar response as found in the metaphase aberration analysis, since short treatment times did not appear to produce any alterations whereas after 8 and 14 hours of recovery, chromatid bridges and fragments showed up.

Molinate also affected the centromeres since with 4 h of exposure and 8 and 14 h of recovery, inactivation of the centromeres ocurred as well as isochromosomes due to transverse rupture of the centromeres. This latter effect appeared at a very low frequency and only with some concentrations (Tables III and IV). However, higher frequencies of chromosomes with inactivated centromeres appeared at higher concentrations of molinate after 8 h of recovery, but at 12 h the frequency decreased at 100 ppm. As the chromosomes with the inactivated centromeres were not involved in normal anaphase kinetics, the alterations would result in aneuploids (Gómez-Arroyo *et al.* 1985).

On the other hand, almost all the molinate concentrations produced multipolar anaphases as consequences of spindle disturbances (Tables III and IV).

The induction of more than one spindle produced cells with different chromosome numbers which were normally lethal for the daughter cells (Brachet 1975).

TABLE III. CHROMOSOME ALTERATIONS (—CONTROL) INDUCED IN ANAPHASE CELLS BY MOLINATE DURING 4 HOURS OF TREATMENT AND 8 HOURS OF RECOVERY

Concentrations ppm	*Total anaphases*	*Abnormal anaphases* %	*Total aberrations (bridges and fragments)* %	*Chromosomes with inactivated centromeres* %	*Isochromosomes* %	*Multipolar anaphases* %
25	377	2.24	2.20	0.70	0	0
50	1 066	2.46	1.17	1.70	0	1.12
100	298	8.43	4.94	4.12	0.24	0.58
2000	C e l l	d e a t h				

TABLE IV. CHROMOSOME ALTERATIONS (—CONTROL) INDUCED IN ANAPHASE CELLS BY MOLINATE WITH 4 HOURS OF TREATMENT AND 14 HOURS OF RECOVERY

Concentrations ppm	*Total anaphases*	*Abnormal anaphases %*	*Total aberrations (bridges and fragments) %*	*Chromosomes with inactivated centromeres %*	*Isochromosomes %*	*Multipolar anaphases %*
25	434	0.43	1.43	0.69	0	0.14
50	2 873	3.45	2.77	1.40	0.08	0.59
100	697	3.74	3.82	0.36	0.34	0.50
200			C e l l d e a t h			

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