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Plant activation of herbicides into environmental mutagens: the waxy reversion bioassay

Recently we reported (MNL 49:40-43; Mutation Res. 31:317) that maize seedlings exposed to atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) contain a potent mutagen and that it is absent in untreated control plants. Atrazine alone is not mutagenic. To evaluate the mutagen-inducing capabilities of field-applied atrazine, we developed a bioassay in which the reversion frequency at the waxy locus in maize pollen grains is used. This bioassay is especially suited for detecting environmental mutagens because (i) reversion at a specific locus can be studied and the genetic effect of a chemical upon the germ cells of a higher eukaryote can be determined, (ii) great numbers of pollen grains can be easily and rapidly scored, and (iii) the mutagen tests can be conducted under field conditions common in agriculture.

An isolation plot was planted with inbred W22 homozygous for the wx-C allele. Field grade atrazine (Aatrex-80W, Ciba Geigy) was applied before emergence at rates of 1, 3, 5, 10, 20, 30 and 50 equivalent lbs/acre (1.12, 3.37, 5.61, 11.22, 22.44, 33.66 and 56.10 equivalent kg/ha) on seven sub-plots. One sub-plot was not treated. Additional control plants were grown for us by G. W. Beadle in his herbicide-free nursery at the University of Chicago. After the plants reached anthesis the tassels were harvested, fixed, and stored in 70% ethanol. Anthers were dissected from unopened florets, the pollen isolated, and scored for Wx or wx according to the method outlined by Nelson (Am. Naturalist 91:331-332).

Data from five sub-plots have been collected (Table 1). The data clearly indicate an increase of over an order of magnitude in the reversion frequency at the wx locus in pollen grains from atrazine treated plants as compared to the control reversion frequency.

Table 1. The Wx reversion frequency of homoallelic inbred W22 exposed to various concentrations of field-applied atrazine.

Concentration of atrazine (equivalent lbs/acre)	Estimated number of gametes	Reversion frequency of Wx (X 10^{-5})
0 Control	514,530	3.11
1	1,017,920	6.19
3	570,710	26.28
5	878,400	29.71
10	993,760	38.74

We believe that these preliminary findings along with previously reported data strongly indicate that a plant-mediated metabolite of atrazine is a potent mutagen to eukaryotes. Also we contend that this bioassay can be easily incorporated with other mutagen test systems for the evaluation of pesticides used in the production of corn. (Partially funded by a D. F. Jones Fellowship, Research Corporation, New York).

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