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Evaluation of Bravo, Phosdrin and Telvar as Possible Environmental Mutagens

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Chemical pollutants present a genetic hazard to man of sufficient importance to warrant an intensive genetic investigation. WUU & GRANT (1966) with atrazine observed chromosome aberrations and mutant seedlings in the C_2 progeny. SUNESON & JONES (1960) reported that when Dalapon was applied to soil as a preemergent weed killer, the developing wheat and barley plants showed malformations, reduced fertility and dwarfs for four generations. Some fungicides have also been found to cause chromosome abnormalities including the production of polyploid nuclei, mutations and malformed offspring. WUU & GRANT (1966, 1967) observed cytological abnormalities in Vicia faba and dwarf plants in Hordeum vulgare with fungicide Dichloran. The fungicide Captan induced chromosome aberrations and produced teratogenic effects on chicken embroys (MALLING & DE SERRES 1970). Data are accumulating to show that pesticides are playing a role in the evolution of plant communities (GRANT 1971).

AHMAD & GRANT (1972) reported that when Vicia faba root tips and pollen mother cells were treated with the insecticide Phosdrin, a greater number of chromosomes was found in the meiotic cells than in the mitotic ones. These authors suggested that lethal sensitivities and mutational susceptibilities to chemicals tend to differ not only from species to species but even between the different tissues of an individual organism. TOMPKINS & GRANT (1972) reported biological effects of an s-triazine insecticide, Menagon, and a herbicide Metobromuron and an aromatic hydrocarbon fungicide, Tetrachloroisophthalonitrile. Their results showed that only Metobromuron had a significant effect on germinability of barley seeds but did not cause any significant effect on chromosomes. MOHANDAS & GRANT (1972) reported cytological abnormalities induced by 2, 4-D and Amitrole. These abnormalities in root tip cells included chromosome bridges, lagging chromosomes and chromatin bodies. WUU & GRANT (1966) reported an interesting case whereby the cytological examination of pollen mother cells of a barley plant from seed treated with a concentration of 500 ppm of the herbicide Lorox showed that all the cells were abnormal in meiotic

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behavior and yet this plant had been capable of surviving to the reproductive stage and was morphologically normal. In another study these authors found that monuron produced 75% and 98% abnormalities in pollen mother cells of two barley plants while the other two were essentially free of chromosomal abnormalities (WUU & GRANT 1967).

Our study was undertaken to evaluate three pesticides for their possible mutagenic properties using barley shoot tips. They were: a fungicide (Bravo), an insecticide (mevinphos) and a herbicide (monuron). The frequency and type of chromosome aberrations were used to estimate the genetic damage. The reduction in seedling height (seedling injury) was used to determine the extent of physiological as well as genetical damage by these pesticides.

MATERIALS AND METHODS

Barley seeds (Carypses), Hordeum vulgare cv. Trent were exposed to three pesticides. Bravo, containing 75% Tetrachloroisopthalonitrile was obtained from the Diamond Shamrock Company. Mevinphos (Phosdrin), containing 100% Dimethyl Phosphate of Methyl 3-hydroxy-cis-crotonate, was obtained from the Shell Chemical Company & monuron (Telvar), containing 80% 3-(p-chlorophenoxyl) - 1, 1-dimethylurea was obtained from the E. I. duPont de Nemours and Co.

Seeds, presoaked in distilled water for 12 hours were then placed in flasks containing 0,250,500 and 1000 ppm active ingredient of each pesticide to be tested in 0.1 M phosphate buffer at pH 6.5. Air was constantly bubbled through the flasks containing 75 seeds in 100 mL of suspensions of various pesticides. After this treatment period, the seeds were rinsed twice with water to remove the chemicals from the surface. Fifty surface-washed seeds from each flasks were planted according to the technique of MYHILL & KONZAK (1967). The remaining seeds were planted on blotters in petri dishes for shoot tip studies. The shoot tips were collected when the length of the tip was 1/2 to 3/4 the length of the seed and fixed in Carnoy's Solution (4 parts 95% ethyl alcohol, 3 parts chloroform and 1 part glacial acetic acid). For each treatment a total of 300 cells were cytologically examined for chromosome aberrations by the aceto-orcein technique. The seedling injury was estimated as percent reduction in seedling height as compared to control and was recorded after 5 days of planting.

RESULTS AND DISCUSSION

The data in Table I show the type and frequency of chromosome aberrations at 0,250,500 and 1000 ppm of the three pesticides. For each treatment, the percent cells showing aberrations is also listed. The results show that the control plants had 5% cells with mitotic

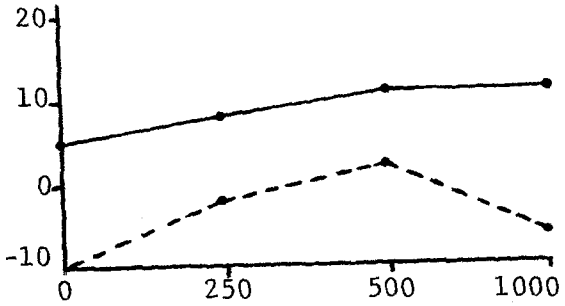
abnormalities, while 250, 500 and 1000 ppm Bravo had 8, 11 and 11% cells with aberrations respectively. This indicates an increase in aberration frequency in treated plants. However, this increase was not statistically significant. The frequency of dicentric bridges likewise showed an increase in treated plants over the control as was the moderate increase in the frequency of multipolar anaphases. Since the increase was not significant it is assumed that Bravo is probably not mutagenic in barley. This observation is also supported by the small differences in the frequency of chromosomal aberrations at various concentrations of the fungicide. Our results are in agreement with those of TOMPKINS & GRANT (1972) who found no significant increase in mitotic abnormalities in root tip cells of barley and Tradescantia at 500, 1000 and 1500 ppm of DAC (tetrachloroisophthalonitrile). The data on seedling injury indicate that there was essentially no difference in height between treated plants and control. In fact there was a slight increase in height, in 250 and 1000 ppm. However, there was a 2% reduction in height at 500 ppm. These results are graphically presented in Fig. 1. The negative seedling injury represents an increase in height rather than a reduction in height. However, this increase was small.

TABLE I

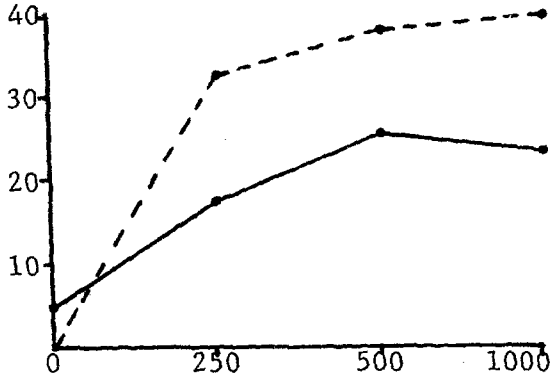
Frequency and Type of Aberrations Induced by Bravo, Mevinphos and Monuron

Name of Chemical	Conc. (ppm)	%Dicentric Bridges	%Multipolar Anaphases & Polyploids	%Cells with Aberr.
Control	0	3	2	5
Bravo	250	3	7	8
	500	11	3	11
	1000	9	5	11
Mevinphos	250	6	12	18*
	500	8	20	26*
	1000	8	16	24*
Monuron	250	2	10	12
	500	8	16	24*
	1000	14	14	26*

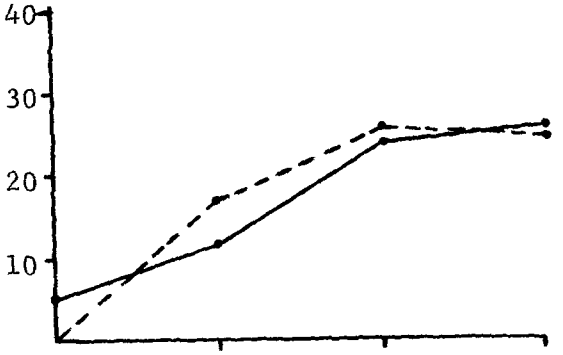
*Comparison of each treatment data with the control data, using individual 2 x 2 contingency Chi Square tests showed that the treatment deviated significantly from the control at the 0.01 level of probability.



Bravo (ppm)
Fig. 1



Mevinphos (ppm)
Fig. 2



Monuron (ppm)

Figures 1 - 3. Percent seedling injury --- --- and percent cells with aberrations —— at various cencentrations of pesticides.

The data for mevinphos are summarized in Table I and in Fig. 2. The percent cells with mitotic irregularities were 5, 18, 26 and 24 at 0,250,500 and 1000 ppm, respectively. The cells with aberrations increased 13 to 19% over control. This increase was significant at .01 level of probability. The low frequency of dicentric bridges indicates that this chemical probably did not cause chromosome breaks. However, a high frequency of multipolar anaphases and polyploid anaphases was observed. AHMAD & GRANT (1972) also reported a highly significant increase in aberrations caused by mevinphos in root tip cells of Vicia faba and Tradescantia. However, these authors reported a low frequency of multipolar anaphases in those plants. The data in Fig. 2 show considerable amount of seedling injury ranging from 33 to 40% caused by mevinphos. Probably this reduction in height is in part due to chromosomal aberrations. It may be noted that there is very little difference in seedling injury as well as in chromosome aberrations between 250 ppm and 1000 ppm. This may be due to the low solubility of this insecticide in water.

Data showing the effect of monuron are also summarized in Table I. The frequency of dicentric bridges indicates that at higher concentrations (500 and 1000 ppm), this chemical caused an increase in chromosome breaks. The percent cells with aberrations in 500 and 1000 ppm were significant at 0.01 levels of probability. WUU & GRANT (1967) reported that two of the four barley plants they studied had 76% and 98% of pollen mother cells with aberrations and the other two were free of abnormalities. As discussed earlier meiotic cells are more sensitive than mitotic cells to chemicals as well as to radiation. The seedling injury caused by monuron was 17.2% at 250 ppm and 25.9% at 500 ppm. This indicates that monuron is slightly toxic to barley.

In our studies mitotic chromosome abnormalities were used as the criteria for mutation induction. The data of KIHLMAN (1966) and EPSTEIN & LEGATOR (1971) showed a marked correlation between the ability to induce chromosome aberrations in plant root tips and mutagenicity.

Three pesticides evaluated for mutagenic properties differed in their effect on barley. Bravo did not cause any seedling injury nor showed any significant effect on chromosomes. Mevinphos, on the other hand increased the frequency of chromosome abnormalities significantly and was also toxic to plants as evidenced by the reduction in height. Monuron also increased the chromosome abnormalities significantly; however, it was only moderately toxic.

It must be pointed out that: (1) dosages used in our studies were generally lower than recommended dosages for pest control, (2) possibility exists that

point mutation may have been caused which is not revealed by chromosome studies, (3) meiotic cells are known to be more sensitive than mitotic cells. In view of these limitations it may be concluded that chemicals must be tested under several different conditions and under different test systems to fully evaluate for their possible mutagenic properties.

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