ABSTRACT

M.Sc. Maryam Ahmed Biology

CYTOLOGICAL EFFECTS OF PESTICIDES ON SOME PLANT SPECIES

The cytological effects of three pesticides, Phosdrin (insecticide), Bladex (herbicide), and Panogen 15 (fungicide), on Tradescantia clone 02 and Vicia faba have been studied. Ethyl methane sulfonate (EMS) and colchicine have been used for comparative purposes. Mitotic and meiotic studies with Phosdrin and Bladex produced the same kinds of cytological abnormalities as EMS. The various types of chromosomal aberrations observed were: fragments, bridges, multipolar anaphases, and lagging chromosomes. The average frequency of chromosomal aberrations induced by both Phosdrin and Bladex deviated significantly from that of control. The cytological abnormalities induced by Panogen 15 were comparable to those produced by colchicine, namely: c-mitoses, multinucleate cells and polyploidy. Two interesting phenomena observed were: differentially stained chromosomes and transverse interconnections between the chromatids. Vicia faba was more susceptible to chromosomal aberrations than Tradescantia. Although all three pesticides induced chromosome aberrations, Panogen 15 was the most injurious to the genomes of both Tradescantia and Vicia faba.

CYTOLOGICAL EFFECTS OF PESTICIDES ON SOME PLANT SPECIES

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INTRODUCTION

Morgan (1914), who introduced Drosophila to genetic research, tried to induce mutations with chemical substances but with little success. It was not until almost two decades later that Muller (1927) first established the mutagenic effects of X-rays on Drosophila. The work with radiation however, was restricted almost entirely to basic research; Muller stated nothing about the possible practical applications of his results. Auerbach and Robson (1944) were the first to achieve positive mutagenic action with a chemical. They established that nitrogen mustard is radiomimetic. Radiomimetic chemicals are those which mimic the most important end effects induced by ionizing radiations in living systems. These include gene and chromosome mutations, carcinostatic and carcinogenic effects and in vitro depolymerization of nucleic acids.

After this interesting beginning, the field of chemical mutagenesis rapidly expanded. The dramatic appearance of Rachel Carson's 'Silent Spring' (1962) awakened the nation to previously little considered deleterious effects of pesticides. Our technology had surged ahead of us. While the value of pesticides in controlling unwanted organisms is of unquestionable economic importance, it is now evident that the use of pesticides has many secondary consequences. The best pesticides are those which kill the pests without affecting the organism.

A number of pesticides have come into common use in the last few years. Studies in progress indicate that a number of pesticides

produce both cytological abnormalities and gene mutations. It is becoming apparent that the action of pesticides is not necessarily restricted to a physiological role: the disturbance of the genetic apparatus may also be a primary function.

Insecticides like DDT, herbicides like 2,4-D, fungicides like methyl mercury compounds, growth regulators like maleic hydrazide, chemosterilants like METEPA, etc., are quite well-known as chemical mutagens. However, there are still a number of pesticides in use, whose effects on plants and other organisms have yet to be determined.

The present study was initiated to determine the cytological effects of three pesticides using Tradescantia clone 02 and Vicia faba as the test materials.

LITERATURE REVIEW

The number of chemical compounds used as pesticides has been continually increasing in recent years. The studies on morphological, physiological and cytological changes to organisms induced by pesticides date back to 1931 (Kostoff, 1931). Kostoff, in carrying out observations on tobacco plants noted that seed set was greatly reduced after the plants had been fumigated with nicotine sulphate. In an examination of the developing meiocytes in the flower buds, he found many chromosome irregularities which he concluded led to the partial sterility of the plants. Subsequently, a number of investigators have made similar observations.

In the following review, the cytogenetic effects of pesticides on living organisms in general, and for plants in particular, are summarized. As colchicine and ethyl methane sulfonate (EMS) have been used as standard mutagens for comparative purposes, these chemicals have also been included in this review. The literature has been considered under five different headings, namely: colchicine, ethyl methane sulfonate, insecticides, herbicides, and fungicides.

I. COLCHICINE

Colchicine was first isolated from Colchicum autumnale, the autumn crocus, by Zeisel in 1883. The alkaloid derived from this plant is isolated from the roots. The empirical formula of colchicine is $C_{22}H_{25}O_6N$.

According to Levan (1938, 1954), the modification of mitotic behaviour induced by colchicine, which he refers to as c-mitosis, consists of "...an inactivation of the spindle apparatus connected with a delay of the division of the centromere" (Levan, 1938). In colchicine treated cells as a result of the lack of centromere activity and a functioning spindle, the division of the centromeric regions is delayed for several hours. It is this extension of the period between the disruption of the nuclear membrane and the division of the regions adjacent to the centromere which is responsible for the increased frequency of mitotic cells observed after treatment with colchicine. The stage delayed by colchicine corresponds to the metaphase of the normal mitosis, and for this reason colchicine is said to cause an "accumulation of metaphases". Nor is there, an anaphase in the usual sense. In the stage corresponding to c-telophase, the chromosomes separate only slightly since the spindle does not function and all of the chromosomes remain in one nucleus, which, consequently, will contain double the normal chromosome number.

In the root tips of Allium cepa, Levan (1938) observed a very high degree of polyploidy after prolonged treatments with colchicine. Thus, a 72 hours treatment gave rise to cells containing as many as 256 chromosomes, which is 16 times the diploid number ($2n = 16$).

II. ETHYL METHANE SULFONATE (EMS)

The monofunctional alkylating agent ethyl methane sulfonate ($CH_3SO_2OC_2H_5$) has been used a number of times as a mutagen for treating both plants and animals.

The first reported biological effect of ethyl methane sulfonate was the inhibition of tumor growth (Haddow and Ross, 1956). Afterwards, the high mutagenicity of this chemical was discovered by Fahmy and Fahmy (1957) in Drosophila melanogaster. Loveless and Howarth (1959) have reported that EMS produces mutations in Salmonella typhimurium and Escherichia coli.

Ehrenberg et al. (1961) tried to compare the genetic effects of ionizing radiations and chemical mutagens. They found that EMS produces the highest percentage of chlorophyll mutations in barley. Ionizing radiations, when compared to EMS and ethyleneimine, produced a greater number of chromosomal aberrations. The authors also indicated that these chemical mutagens have an affinity for certain regions of the gene material, that is deoxyribonucleic acid (DNA), and so are more restricted in their effect than are ionizing radiations.

Arnason et al. (1962), also working on barley, have confirmed that EMS is quite effective in causing mutations affecting plastids and their pigments.

The mutagenic effects of EMS on Vicia faba and barley were studied by Moutschen-Dahmen et al. in 1963. They treated dry seeds of Vicia faba and barley with EMS. They found that this compound had practically no chromosome breaking effect. However, addition of copper and zinc sulfates enhanced chromosomal aberrations. Cu^{2+} at pH 7.8 and Zn^{2+} at pH 7.2 were most efficient. The observed effects with Cu^{2+} and Zn^{2+} were proportional to the dose of EMS. Chromatid breaks were quite common.

Froese-Gertzen et al. (1964) compared the biological effects of EMS and X-rays in barley. The criteria they used were seedling injury, plant survival, mitotic and meiotic chromosome aberrations, germinal mutations, and the frequency of chimeras. The level of injury to seedling growth, survival, and the proportion of albino mutations produced by the two treatments were quite similar. The frequency of chlorophyll deficient mutations was higher in EMS treated plants than in X-rayed ones showing the greater specificity of EMS. The frequency of chromosome bridges and fragments induced by radiation was considerably higher when compared with EMS. Fertility was low after EMS treatment.

The chromosomes of Vicia faba can be distinguished from one another and are known to possess heterochromatic regions. Using these facts, Natarajan and Upadhya (1964) determined the localization points of chromosome breakage after EMS and hydroxylamine treatment. The breakages induced by EMS and hydroxylamine were very specific and localized, and by either of the chemicals, it was found that the same regions in the chromosome are broken. These breakage points coincide with some of the known heterochromatic regions of the chromosomes. Centromeric regions were preferentially affected, as revealed by centric breaks as well as the inactivation of centromeric activity.

Rao and Natarajan (1965) tested the mutagenicity of different alkyl alkane sulfonates in barley. Methylating agents were less mutagenic and more toxic than other agents used in their study.

Ethylating agents were highly mutagenic. The frequency of mitotic and meiotic aberrations induced by EMS was quite low.

It has been shown that a temperature difference of 10°C during the recovery period can have a considerable effect on the mutation frequencies (Satpathy and Arnason, 1969). The highest mutation frequencies were observed in experiments with high temperatures during the recovery period. The authors suggest that this may be due to interference with the recovery processes, or to speeding up of continuing alkylation by free EMS still retained in the nucleus.

Grinikh (1969) has studied the types of chromosomal aberrations induced by EMS in Crepis capillaris by subjecting seeds to different physiological conditions. After using high concentrations (1 g/100 ml.) of EMS for 3-5 hours, he noted that the frequency of chromosomal aberrations in metaphases of the first mitosis was not significantly higher than that of control. The addition of Cu-ions, the variation of pH, and the time of fixation, appeared to have no effect on the frequency of chromosomal aberrations. Neither EMS, nor its secondary reaction products acted on the chromosomes at the G-stage, but did so at the S-stage.

Swaminathan et al. (1962), have studied the frequency of chromosome aberrations and the spectrum of mutations induced by EMS in barley and wheat. They used diploid and polyploid seeds of wheat and barley and treated them with different concentrations of EMS. They observed decreased survival, occurrence of chlorophyll deficient streaks in the leaves and plant sterility. Chromosome and chromatid breaks

occurred but interchanges were few. A greater number of breaks were noted at primary and secondary constriction regions. Chlorophyll mutations were carried into the second generation. They concluded from their study that polyploids were less affected than diploids, and that response to EMS treatment may vary from species to species. In a more recent study on a hexaploid dwarf wheat, Vaurughese and Swaminathan (1970) have compared the spectrum and the frequency of mutations induced by gamma rays and EMS. They found that EMS was capable of inducing a wide range of mutations with a higher frequency than gamma rays.

III. INSECTICIDES

A number of insecticides have been used to protect plants against insects. They belong to four chemical groups: (1) Chlorinated hydrocarbons, (2) Cyclodiens, (3) Carbamates, and (4) Organophosphates. The cytogenetic effects of insecticides belonging to these different groups will be reviewed.

1. Chlorinated hydrocarbons

All the chlorinated hydrocarbon insecticides in common use share an amazing persistence in soils, vegetable matter, in domestic and wild animals, and in man.

Vaarama (1947) has studied the influence of DDT (dichloro-diphenyl trichloro-ethane) upon plant mitosis. Saturated solutions of DDT in tap water had only a weak c-mitotic effect, probably because of the poor water solubility of DDT. By treating root tips of Allium cepa and Trigonella foenum graecum with a mixture of DDT and ethyl alcohol, Vaarama observed a series of cytological

abnormalities which included partial c-mitosis, multipolar spindles, polyploid cells, chromatid breaks, and chromatin eliminations in Allium cepa. He noted intensive stickiness of the chromosomes and nucleolar substances which entirely covered the metaphase chromosomes in Trigonella foenum graecum. Scholes (1955b), in her studies with DDT in Allium cepa, reported significant delays in metaphase and anaphase and the shortening of the chromosomes in metaphase. More recently Ficsor et al. (1970) have tested the mutagenicity of DDT, nitrosoguanidine and Captan (N-trichloromethylmercapto-4-cyclohexane-1, 2-dicarboximide) on Zea mays. Incontrast to previous results, this study showed that of the three compounds tested, only nitrosoguanidine was mutagenic in maize.

Hexachlorocyclohexane (HCH) is a mixture of isomers produced by chlorinating benzene with six atoms of chlorine; but since the aromatic character of the ring disappears, it is entirely incorrect to call the product benzene hexachloride. Unfortunately, the use of the term benzene hexachloride and its abbreviation, BHC, is still quite common, but should be abandoned in favour of the term hexachlorocyclohexane (HCH).

Kostoff (1948) has studied the effect of insecticides containing hexachlorocyclohexane on seedlings of Zea mays, Triticum vulgare, T. durum, T. monococcum, T. compactum, Secale cereale, Helianthus annuus, Crepis capillaris, Vicia faba, V. sativa, and Brassica nigra.

C-mitosis and polyploidy were the common phenomena observed. Chromosome multiplication lead to an increase in the size and

occasionally in the number of nuclei, and further to the increase in the size of the cells. This cell expansion resulted in swelling of the roots, stems, and coleoptyles. However, chromosome and chromatid breaks were quite rare.

Datta, in 1966, confirmed the previous findings that gammexane causes chromosomal derangements and polyploidy. He used saturated solutions of gammexane on Urginea coromandeliana and noted extreme stickiness of the chromosomes and clumped metaphases. However, dilution of the saturated solution caused polyploidy. Chromatid bridges, peripheral arrangement of the chromosomes, and full contraction of metaphase chromosomes were also observed.

"Bug master" is an insect killer for the home. It vaporizes "Lindane crystals"--"100% gamma isomer of benzene hexachloride". Sax and Sax (1968) found no radiomimetic effect of the unheated crystals when placed in a closed container with the germinating seeds of Allium cepa. The "Lindane crystals" are not soluble in water. Sax and Sax dissolved the crystals in alcohol at concentrations of 1/10,000 to 1/80,000 and found that root growth was not retarded. At 1/80,000 anaphase frequency was normal, but the aberration frequency was high. They also tested the radiomimetic effect of two commercial sprays of Lindane: the liquid form "ortho" and the powder form. The results showed that at comparable concentrations of the gamma isomer, the three insecticides had comparable effects.

2. Cyclodienes

Insecticides belonging to chlorinated hydrocarbons and cyclodienes have been used by Scholes (1955a) for cytogenetic studies in Allium cepa. Her experiments with chlordane and toxaphane show that these two chlorinated hydrocarbons act as weak narcotics in causing a delay in the appearance of the spindle. The author also tested the effects of aldrin, dieldrin, isodrin and endrin which belong to the cyclodienes group, and found that these compounds are less phytotoxic and less likely to cause cytological derangements than are chlordane and toxaphane.

The effects of Endrin (hexachloro-octahydro-endo-dimethanonaphthalene) on somatic cells of Vicia faba, and mitotic and meiotic cells of barley, have been tested by Wuu and Grant (1966a, 1967a, 1967b). This insecticide has been found to be quite mutagenic in Vicia faba and barley. Chromosome irregularities increased with an increase in treatment period and concentration of the pesticides in somatic cells of Vicia faba and barley. The same chemical had a weak effect in producing chromosome aberrations in the germ cells of barley.

3. Carbamates

The effects of isopropyl phenyl carbamate (IPC) on rye and onion were studied by Doxey (1949). The effects of IPC resembled, in many respects, those produced by colchicine. Interference with centromere division, and spindle suppression leading to polyploidy, were common in both rye and onion. Multipolar spindles and multinucleate cells were seen most frequently in onion. Chromatid

fragmentation took place only in rye. Root tips developed club shaped swellings characteristically produced by mitotic poisons. The author suggested that carbamates interfere with purine metabolism, but no concrete evidence supporting this idea was provided.

Sevin (1-naphthyl-N-methyl carbamate) causes chromosome fragmentation and other abnormalities in root and pollen mother cells of Vicia faba and barley (Amer, 1965; Amer and Farah, 1968; Wuu and Grant, 1966a, 1967a, 1967b). However, Vicia faba is more susceptible to Sevin than is barley (Wuu and Grant, 1966a, 1967a, 1967b). There was little correlation between the percentage of cells found with chromosome abnormalities for treatments in the C_1 generation and those found in the C_2 generation.

4. Organophosphates

Organophosphates have been used by a number of workers to determine their mutagenic effects on plants as well as animals.

The cytological effects of the insecticide Systox [0,0-Diethyl O-2(ethylthio) ethyl phosphorothioate]--an organophosphate--on the primary roots of Vicia faba, and its mutagenic effects on Arabidopsis thaliana, have been studied by Veleminsky and Gichner (1963). A 0.02% water solution of Systox was used for treatment periods of three, six and nine hours. No inhibition of mitosis was produced. Chromosomal aberrations such as fragments, bridges, and lagging chromosomes were noticed. Concentrations of 0.2% and 1% Systox for 6, 48 and 72 hours on seeds of Arabidopsis

produced no chlorophyll or other mutations in the M_1 or M_2 generations.

Phosphomidon (2-chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate), another organophosphate insecticide, acts as a chromosome breaking agent in Vicia faba and barley (Wuu and Grant, 1966a, 1967a, 1967b).

"Vapona" is a household insecticide which kills insects by releasing a toxic vapour. The active ingredient is 2,2-dichlorovinyl dimethyl phosphate. The frequency of chromosome aberrations increases with the size of the Vapona strip used. In no case was there any retardation of root growth or mitotic frequency, but at higher concentrations there was some polyploidy (Sax and Sax, 1968).

Gibson and Beinhart (1969) noticed that Demeton [O-(2-ethylmercapto) ethyl O,O-diethyl thiophosphate] causes meiotic abnormalities in the sporocyte tissue of clover plants (Trifolium spp.). The abnormalities observed were clumping of chromosomes at anaphase, and terminal associations of chromosomes at anaphase.

Mitotic and meiotic effects of two organophosphorous systemic insecticides, viz., Dimecron-100 (Dimethyl 2-chloro-2-diethyl carbamoyl-1-methyl vinyl phosphate) and Rogar-40 (N-monomethylamide of O,O-dimethyldithiophosphorylacetic acid) used in different concentrations, were studied in Vicia faba by Reddy and Rao (1969). Chromosome and chromatid breaks, dot-deletions, fragments, and anaphase bridges, were noticed in both metaphase and anaphase stages of mitosis. In meiosis, aberrations such as fragments, ring chromosomes, anaphase bridges, laggards, and tetraploidy, were observed. On the

whole, Dimecron-100 was more radiomimetic than Rogar-40.

IV. HERBICIDES

Herbicides have been widely used in the production of field crops. Many herbicides are known to produce mutations (Unrau, 1953, 1954; Mühling et al., 1960; Wuu and Grant, 1966a, 1967a, 1967b). A better understanding of the cytogenetic effects of herbicides would give some knowledge of any potential harmful effects to the crop species and other organisms.

1. Phenoxy acetic compounds

The phenoxy herbicides 2,4-D (2,4-Dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-Trichlorophenoxyacetic acid) have received wide publicity over the past year since they have been widely used as defoliants in Vietnam. These herbicides produce both morphological as well as cytogenetic abnormalities.

The induction of morphological abnormalities in 2,4-D treated plants has been reported for barley (Derscheid et al., 1952; Smith and Harrison, 1962), corn (Rossman and Sprague, 1949) and beans (Pridham, 1947). Derscheid et al. (1952) observed both yield reduction and spike malformations in treated barley seedlings, but neither of these characters were transmitted to the succeeding generation. In contrast, Unrau (1953, 1954) showed that 2,4-D induces heritable changes in awning, earliness, and stature of barley and wheat plants.

Croker (1953), investigating the effects of 2,4-D and 2,4,5-T on mitosis in Allium cepa, noted both physiological and structural abnormalities of the chromosomes such as stickiness, condensation of chromosomes, and chromatid breaks.

The effects of five phenoxy hormonic herbicides on the staminal hair cells of Tradescantia, the stipular cells of Vicia faba, the petal cells of Tradescantia and isolated root tip cells of Triticum, were studied by Sawamura (1964). The herbicides he used were 2,4-D, 2,4,5-T, 2,5-D (2,5-Dichlorophenoxy acetic acid), MCP (2-methyl-4-chlorophenoxy acetic acid) and SES (2,4-Dichlorophenoxyethylsulfate). Their effects on cells in the metabolic as well as in the mitotic state were studied. Abnormal mitoses were induced by these chemicals. Chromosome bridges due to stickiness, retardation of chromosome movements in anaphase, binucleate and multinucleate cells with multi-septa, the formation of incomplete cell walls, and an impediment in the differentiation of meristematic tissues, were observed. The effects of these herbicides on the mitotic cells revealed the close connection between herbicidal activity and plant malformations.

Embutox E [4-(2,4-dichlorophenoxy)-butyric acid] is also a phenoxy herbicide. A study of the mitotic and meiotic effects of this herbicide on Vicia faba and barley has been carried out by Wuu and Grant (1966a, 1967a, 1967b). Embutox inhibited seed germination in barley and caused abnormal growth of seedlings in both barley and Vicia faba. It also caused chromosome fragmentation in the somatic and

germ cells of barley.

Using a labelling technique, MacLeod (1969) has shown that 2,4,5-trichlorophenoxy acetic acid lengthened the duration of the mitotic cycle by extending the S and G_2 phases, and caused an initial increase in the rate of DNA synthesis and a decrease in the number of cells in S-phase.

2. Triazines

A number of herbicides belong to the S-triazine group. Various concentrations of TEM [2,4,6-(tri-ethyleneimino)-1,3,5-triazine] have been used to treat root tips of Allium cepa, Vicia faba and microspores of Tradescantia. Bridges and fragments were observed in both Allium and Vicia faba (Wakonig and Arnason, 1959). TEM showed no specificity for heterochromatic regions in Vicia faba as reported earlier for some chemicals.

Two herbicides, CAT (2-chloro-4,6-bisethyl-amino-s-triazine) and ATA (amino triazol), have been used by Sawamura (1965) on various cells of Tradescantia, Vicia and Allium. CAT interfered with mitosis only weakly. In contrast, ATA produced multinucleate cells with multisepta, cells with double the number of chromosomes, and binucleate cells, both in Tradescantia and Allium. When the cells became necrotic, they first coagulated and then liquified.

Three triazine herbicides, Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), Cytrol (3-amino-1,2,4-triazole) and Simazine 2-chloro-4,6-bis(ethylamino)-s-triazine , have been used

by Wuu and Grant (1966a, 1967a, 1967b) to treat seeds of barley and seedlings of Vicia faba. These herbicides did not produce any morphological abnormalities in barley, except Cytrol which inhibited seed germination. All three herbicides caused cytological aberrations in both barley and Vicia faba. Vicia faba seemed to be more susceptible to these chemicals than barley. The most common aberrations observed were metaphase and anaphase fragments, and anaphase bridges. Other abnormalities, such as multipolar anaphases, incomplete chromosome breaks, lagging chromosomes, and telophase bridges, were also observed but less frequently. Cytrol was the most effective chromosome breaking agent of the three. From barley seeds treated with Simazine and Atrazine, some of the mutations observed in the C_1 generation plants were persistent, and were transmitted to the succeeding (C_2) generation plants. Simazine and Atrazine have also caused chromosomal aberrations in the meiotic cells of barley.

Liang et al. (1967) have studied the cytogenetic effects and responses of agronomic characters in grain sorghum (Sorghum vulgare) following Atrazine application. The affected microsporocytes showed a number of chromosomal abnormalities including: multinucleate cells, bridges, aneuploidy, and micronuclei. However, no apparent relationship existed between agronomic performance and cytogenetic response following Atrazine application.

Simazine and Atrazine did not affect germination, plant height or frequency of chlorophyll mutations in barley (Stroyev, 1968),

confirming Wuu and Grant's results. The types of chromosomal rearrangements caused by Simazine resembled those produced by maleic hydrazide (Stroyev, 1968).

3. Substituted urea

Monuron [3-(p-chlorophenyl)-1-1-dimethylurea], also known as CMU, is a substituted urea herbicide. After treatment with CMU, chromosome bridges, suppression of cell plate growth, and binucleate cell formation were observed in the hair cells of Tradescantia, but the action of CMU on the metaphase and anaphase spindle was rather weak (Sawamura, 1965). When tested on barley and Vicia faba, Monuron was found highly radiomimetic (Wuu and Grant, 1966a, 1967a, 1967b).

Lorox, which contains 50% 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea as the active ingredient, produces very striking cytological changes in the meiotic cells of barley (Wuu and Grant, 1966b). Stickiness among the chromosomes, incomplete furrowing of cytoplasm, "chromatin bodies" in the cytoplasm, persistent chromosome bridges, and asynchronous and unequal division of pollen mother cells, were observed. Wuu and Grant (1966b) attributed the formation of "chromatin bodies" to the extreme stickiness of the chromosomes, possibly caused by depolymerization of DNA molecules.

4. Chloroaniline

Herbicides belonging to the chloroaniline group have been

studied by Gentner and Burk (1968) and Prasad and Pramer (1969). Nitralin (4-methyl-sulphonyl-2,6-dinitro-N,N-dipropylaniline) caused digitate and globose swellings in the region of active cell division in Zea mays root tips. A cytological examination of the affected area showed that this herbicide prevented: (1) cell wall formation, (2) enlargement of cells, and (3) extensive replication of cells (Gentner and Burk, 1968).

Propanil (3',4'-Dichloropropionanilide), a herbicide used on rice fields to control weeds, and its two degradation products have been tested for their cytogenetic effects on Allium cepa (Prasad and Pramer, 1969). Propanil was more toxic than were the other two compounds. Stickiness of the chromosomes and anaphase bridges were observed.

5. Phenyl carbamates

Carbamate herbicides have been tested on more than 20 species of plants in such diverse groups as gymnosperms, monocotyledons, and dicotyledons. The principal effects are rapid inhibition of cell activity, contraction of chromosomes, and polyploidy (Ennis, 1948). All these abnormalities indicate a "colchicine effect".

Canvin and Friesen (1959) have used the herbicide IPC (isopropyl N-phenyl carbamate) on barley and peas. The herbicide "Avadex" (2,3-dichloroallyl diisopropyl thiolcarbamate) has been used by Morrison (1962) on several crops. From such studies, these and other workers have come to the conclusion that dicotyledons are

more resistant to carbamate herbicides than are monocotyledons.

Storey et al. (1968) have suggested that carbamate herbicides, because of their favourable mode of action on chromosomes to produce polyploid cells, could be used as new tools for cytological studies.

6. Chlorinated benzene compounds

Dicamba (2-methoxy-3,6-dichlorobenzoic acid) is a chlorinated benzoic acid herbicide. It is used to control wild buckwheat (Polygonum convolvulus), Tartary buckwheat (Fagopyrum tataricum), and green smart wheat (Polygonum scabrum) which are not controlled by 2,4-D or MCPA. Both field and laboratory experiments have indicated that the herbicidal activity was strongest in the region of greatest meristematic activity at the time of spraying. The time of treatment and the dosage are very important factors in weed control. The morphological, as well as the mitotic abnormalities, resembled those caused by 2,4-D. However, it is significant that mitosis was disturbed by as little as 10 p.p.m. of Dicamba whereas to obtain similar effects with 2,4-D required 40 p.p.m. of 2,4-D (Friesen et al., 1964).

Another chlorinated benzoic acid herbicide, Banvel-D (2-methoxy-3,6-dichlorobenzoic acid and dimethylamine salts of related acids), has been tested on barley by Wuu and Grant (1966a, 1967a, 1967b). Banvel-D severely affected seedling root growth, causing a high frequency of chromosomal aberrations in the C_1 generation, although the frequency

decreased in the C_2 generation. Banvel-D also caused meiotic aberrations in barley sporocytes.

A number of other herbicides belonging to different chemical groups have also been reported to be mutagenic and radiomimetic in barley, Vicia faba and Allium cepa (Sawamura, 1965; Wuu and Grant, 1966a, 1967a, 1967b), but are not discussed here as they are not pertinent to the present study.

7. Phenolic compounds

A comparative study of a number of phenolic compounds used as herbicides has been undertaken by Mühling et al. (1960). 2,4-dichlorophenol, 2,4-dinitrophenol, and phenol were tested on pea seedlings (Pisum sativum). These compounds had two clearly distinguishable effects on mitosis: (1) spindle inhibition resulting in chromosome configurations very similar to those produced by colchicine, and (2) ultimate inhibition of the onset of mitosis. In addition, the authors also indicated that the compounds interfered with the transition of the chromosomes from late prophase to prometaphase. Chromosome fragmentation occasionally occurred. Certain morphological changes were also observed including cessation of growth of the primary meristem, the occurrence of a tumor-like swelling, and stimulation of secondary root production.

DNOSBP (4,6-Dinitro-o-secondary butylphenol) and PCP (Pentachlorophenol) are two non-hormonic herbicides (Sawamura, 1965). DNOSBP is highly toxic even at very low concentrations. It acts as a typical contact poison in Tradescantia staminal hair cells and in stipular

cells of Vicia faba. PCP affects mitosis at the cellular level and causes a number of chromosomal abnormalities, particularly chromosome bridges, fragments, and polyploidy.

A number of phenolic herbicides and their degradation products such as O-nitrophenol, p-nitrophenol, p-chlorophenol, 2,4-dichlorophenol, pentachlorophenol, α and β naphthol, and 2,4-dichloronaphthol, have been used in mitotic and meiotic studies on Vicia faba (Amer and Ali, 1968, 1969). All the phenols caused a considerable decrease in the mitotic index of V. faba when compared with the control. Disturbed metaphases and anaphases were the most common types of anomalies induced by all the phenols, which included marked contraction of the chromosomes, stickiness, lagging chromosomes, anaphase bridges and fragmentation. Cases of cytomixis were sometimes observed. Meiotic effects included all of the above-mentioned abnormalities except that fragmentation of the chromosomes was quite rare. Of all the phenolic herbicides tested p-nitrophenol was the most toxic. None of the phenols affected pollen viability.

8. Maleic hydrazide

An enormous amount of work has been done on the cytological effects of this compound, hence it has been dealt with individually.

Maleic hydrazide (1,2-dihydropyridazine-3,6-dione) is a synthetic plant growth regulator, and has also been used as a herbicide in agriculture. Indications that maleic hydrazide (MH) is potentially dangerous have been appearing ever since its growth-

regulating properties were discovered in 1949. In 1950, Greulach and Atchison reported the growth and division inhibiting property of MH in root tips of Allium cepa. They suggested that low concentrations inhibit cell division but not cell enlargement, while high concentrations inhibit both functions. In 1951, Darlington and McLeish showed for the first time, that this compound acts as a chromosome breaking agent. It caused chromosome breaks and sister reunion of chromatids at the point of breakage. MH has also been shown to act specifically on heterochromatic regions in Vicia faba (McLeish, 1952). Various concentrations of MH in treatments of Pisum sativum root tips gave parallel results, thus, substantiating the previous reports (Compton, 1952).

Anatomical and cytological studies of barley plants sprayed with MH showed effects of necrosis, hypertrophy, and inhibition of growth. It was discovered that MH has its initial and most deleterious effects on meristematic tissues and tissues that are undergoing rapid differentiation (Gifford, 1956).

A comparison of chromosome breakage induced by MH, its derivatives, and X-rays in four varieties of maize has been carried out by Graf (1957). The breaks caused by MH and its derivatives are localized, whereas those by X-rays are random. These results confirm those of Darlington and McLeish for Vicia faba and Compton for Pisum sativum.

Radiomimetic and mutagenic properties of MH were studied in the C_1, C_2 and C_3 generations of a highly inbred variety of tomato by Grant and Harney (1960). Morphological abnormalities included

a number of mutant seedling plants in both C_1 and C_2 generations. The percentage of abnormalities was proportional to the concentration of the chemical and the duration of treatment in the C_1 generation. In the C_2 generation, however, no correlation was observed. Dwarf, rogue, bushy, mottled and other abnormal forms of seedling plants were observed in the C_2 generation. The chromosomal abnormalities, included anaphase bridges, bridges plus fragments, fragments and multi-polar anaphases.

When testing for mutagenicity in barley, Arnason et al. (1962) found that barley is quite resistant to MH. MH produced very few mutations in the embryos of barley seeds.

MH has also been reported to be a carcinogenic agent in mice (Epstein et al., 1967). Although MH is not highly carcinogenic, it may be a hazard because of its extensive use as a herbicide.

When tested for radiomimetic activity in Rhoeo discolor, MH produced lagging chromosomes, bridges, ring chromosomes, and fragments, in the pollen mother cells (Ammini, 1968).

Stroyev (1968) reported that in barley, low concentrations of MH increased the frequency of chromosomal aberrations, whereas high concentrations suppressed cell division completely.

V. FUNGICIDES

A number of fungicides have also been reported to cause various chromosome abnormalities.

1. Chlorinated hydrocarbons

The mitotic effects of a fungicide containing chloranil (tetrachloro-p-benzoquinone) as an active ingredient have been observed in Allium cepa L. and Vicia faba, respectively (Yakar, 1952). The purchased seeds of Vicia faba had a fine, yellowish powder coating. Enquiry revealed that they had been dusted with a commercial fungicide, whose active ingredient was chloranil. The seedlings of Vicia faba which germinated from these seeds were badly deformed. Cytological examination showed a variety of mitotic abnormalities including multipolar anaphases, bridges, multinucleate and polyploid cells, and occasionally lagging chromosomes (Yakar, 1952).

Cytological abnormalities were also found in chloranil treated onion root tip cells. Extreme contraction of chromosomes, c-mitosis, chromosome bridges, and lobate nuclei, were observed (Yakar, 1952).

The effect of Botran (2,6-dichloro-4-nitroaniline) on Vicia faba and barley was investigated by Wuu and Grant (1966a, 1967a, 1967b). This fungicide produced chromosome fragments and bridges, predominantly; other aberrations were quite rare. The most striking effect was the high percentage (11.32%) of pollen mother cells in the C_2 generation with chromosome abnormalities as compared to 1.26% in the C_1 generation.

2. Thiocarbamates

Ferbam (ferric dimethyl dithiocarbamate) is used as a fungicide. Prasad and Pramer (1968) described the mutagenic activity of Ferbam

when applied to Aspergillus niger and to growing Allium cepa roots. Ferbam induced color mutants and back mutations in A. niger. In Allium cepa, there was a high frequency of chromosomal aberrations which included adhesion of chromatids, endoploidy, fragmentation, bridges due to adhesion, and dicentric chromatids.

3. Miscellaneous

The treatment of barley seed with five fungicides: Brassicol (pentachloronitrobenzene), Ceresan Wet (containing 2.5% mercury in the form of methoxyethyl mercury chloride), Thiram (tetramethyl thiuram disulphide) and Ziram (zinc dimethyl-dithiocarbamate) induced both morphological and cytological abnormalities. Ziram inhibited seed germination and reduced the height of seedlings. Almost all of these fungicides induced some abnormalities in the chromosomes, the most frequent of which were fragments, bridges, and lagging chromosomes. Other alterations included aneuploidy, unequal distribution of chromosomes, tripolar spindles, micronuclei, and spindle inhibition (George, Aulakh and Deshi, 1970).

4. Mercuric fungicides

The mercuric fungicides have recently received wide publicity after reports of birds dying from eating treated seeds, and of fish containing quantities of mercury harmful to human health. The increase in the mercury content of wild birds has been shown to coincide remarkably well with the rising use of organic mercuric fungicides. But the mercury problem is complicated by pollution of streams and lakes by some industries.

The high mercury content in fresh water fishes, has been traced to industrial sources of mercury discharge. This environmental circulation, particularly of methyl mercury compounds, has initiated concern about the detrimental effects to human health which may be occurring.

In 1937, Sass made some histological and cytological studies of ethyl mercury phosphates. Seedlings of corn developed thickened leaf primordia and other morphological irregularities. Cell division was inhibited and cells became very large in size. Multinucleate cells, with 'micronuclei' to very large 'giant nuclei', were observed. Kostoff (1939) using another fungicide "Granosan" (2 parts of CH_3CH_2HgCl + 98 parts of alcohol), verified the c-mitotic action by organic mercury compounds.

Seedlings of Raphanus and Zea were treated with phenyl mercuric hydroxide and basic phenyl mercuric nitrate (MacFarlane, 1950). Leaves with enlarged stomata, sectorial chlorophyll mutations, pollen sterility, polyploid nuclei, sticky chromosomes and fragments, were observed.

Fahmy and Fahmy (1951) and Ramel and Magnusson (1969) have carried out some interesting work on the mutagenic and cytological effects of various organic and inorganic mercurial compounds on Drosophila melanogaster.

Ramel (1969) and Fiskesjö (1969) have studied the genetic effects of organic mercury compounds on Allium roots. A qualitative and quantitative survey of the effects of mercuric compounds on the mitotic apparatus and on the chromosomes was obtained. The substances

tested included pure organic mercury compounds as well as the fungicide Panogen, containing methyl mercury dicyandiamide. The predominant cytological effect of the mercury compounds is c-mitotic action, largely resembling that of colchicine. While mercurials are considerably more toxic than colchicine, the dose range which gives rise to complete c-mitosis is much more restricted than for colchicine. As a result of c-mitotic action, polyploid as well as aneuploid cells develop after mercurial treatments.

All mercury compounds have been shown to cause c-mitotic tumors and hook-like growths in root tips. Chromosome fragmentation was caused by phenyl mercury and to a minor degree by methyl mercury (Ramel, 1969).

Fiskesjö (1969) used: (1) a commercial fungicide with 'betoxin' as the active ingredient, (2) an organic mercury compound and (3) four chemically related mercurials on Allium cepa to test the c-mitotic action of these compounds. His results parallelled those of Ramel (1969). With decreasing concentrations the following reactions were observed: chromosome stickiness, differential staining of heterochromatic regions, c-mitosis, and c-tumors.

In further studies, Fiskesjö (1970) reported the action of two organic mercury compounds, namely, MMC (methyl mercury chloride) and MOEMC (methoxy ethyl mercury chloride) on human leukocyte cultures. Toxicity and c-mitotic action were observed. In comparison with the Allium test system (Fiskesjö, 1969), the compounds usually had somewhat lower threshold values. Recently organic mercury has been identified

as the cause of poisoning in humans and hogs (Curley et al., 1971).

In conclusion, it can be said unassailably that some of the pesticides used are mutagenic and radiomimetic. Some of them can cause mutations even at recommended dosages. Great efforts have been made to understand the mutagenic effects of DDT, 2,4-D, maleic hydrazide, and more recently of mercuric fungicides. However, there are a large number of pesticides which are in common use, and more appearing on the market daily, which remain untested. It is the object of this thesis to provide cytological information on some previously untested pesticides in order to come to a better understanding of the biological nature of these agents.

MATERIALS AND METHODS

1. Plants

Tradescantia clone 02 and Vicia faba (broad bean) have been used as the experimental plants in this study. Plants of Tradescantia clone 02 were obtained from the Brookhaven National Laboratory, Upton, New York. Seeds of broad bean of the cultivar known as Seville Long Pod were used. The broad bean seeds were obtained from the W. H. Perron Seed Company in Montreal. Root tip and pollen mother cell studies were carried out with these plants as they possess large chromosomes which are suitable for cytological analysis.

2. Pesticides

Three pesticides have been used:

a) Phosdrin: ($C_7H_{13}O_6P$) is the Alpha isomer of 2-carbomethoxy-1-methylvinyl dimethyl phosphate (100% active ingredient). It is a stable liquid insecticide, miscible with water. Phosdrin is used to control certain economically important insects and mites which attack the principal field, forage, vegetable, and fruit crops. It has been provided by Shell Canada Limited.

b) Bladex: [2-(4-chloro-6-ethylamino-s-triazin-2-ylamino)-2-methylpropionitrile]. Bladex 80% WP is a selective preemergence herbicide. The plants controlled by Bladex are grasses, broad leaf weeds in both field and sweet corn. Its action is mainly through the roots. Water solubility is 160 p.p.m. at 23°C. It has been provided by Shell Canada Limited.

c) Panogen 15: (Methyl mercury dicyandiamide). Panogen is the original Liquid Mercurial Seed Treatment Fungicide. The active ingredient is methyl mercury dicyandiamide. 4.549 litres of Panogen 15 contains 105.1947 ml. of methyl mercury dicyandiamide. It is a stable liquid solution, miscible with H_2O. It is a seed treatment fungicide for cereal grains, flax, soybeans, peas, rape, safflower and many other seeds. Panogen is reported to help eliminate seed borne diseases, prevent seed borne seedling blight, and help in the suppression of weeds. Panogen acts both by direct contact and by vapour action. It is labelled POISONOUS, and recommended dosage differs from plant to plant. It is a product of the Morton Chemical Company of Canada.

3. Preparation of solutions

All the solutions used were freshly prepared. The concentration was calculated on a weight basis in parts per million (p.p.m.) of the active ingredients for each chemical dissolved in 1,000 ml. of distilled water. All the solutions were adjusted to pH 7 at room temperature (approximately $24^{o}C$).

4. Treatment of Tradescantia and Vicia faba root tips

To get a large number of root tips, plants were propagated by means of cuttings. The ends of the stems were dipped in 'rooting hormone' (indolylbutyric acid) and planted in flats containing vermiculite. The flats were provided bottom heat. Twenty days later good roots developed. These plants were used for treatment.

Seeds of Vicia faba were soaked in water for 24 hours prior to planting. They were planted in pots containing soil rather than vermiculite. Twenty day old seedlings were treated with the pesticides.

For treatment, of both Tradescantia and Vicia faba plants the vermiculite or soil was washed thoroughly from the roots and each plant placed in a freshly prepared solution of the pesticide to be tested in a 300 ml. beaker. Each beaker was covered both top and sides with heavy black paper and a series of beakers was aerated artificially by means of an air pump. In order to maintain a constant environment for testing the effects of the different pesticides on the seedlings, the entire set up, as described above, was maintained in a growth chamber. The environmental conditions inside the chamber were as follows:

Day temperature..... 23.8°C

Night temperature.... 18.3°C

Humidity..... 70°C

Photoperiod..... 16 hours light

After the designated period of treatment, the solution in each beaker was replaced by tap water. The immersed part of each seedling was thoroughly washed in tap water and then replaced in the beaker for a recovery period of 24 hours under the same environmental conditions.

5. Root tip squashes

Root tips were fixed in fresh Carnoy's fixative (three parts absolute ethyl alcohol:1 part glacial acetic acid) for 24 hours. Then the root tips were stained by the Feulgen technique. After staining, V. faba root tips were treated with 4% pectinase for 20 minutes to soften the tissue; those of Tradescantia for 40 minutes as the root tips of the latter have a thick root cap. The root tips were washed in distilled water and stored temporarily in tap water. They were squashed in 45% acetic acid.

6. Spray treatment of Vicia faba plants

Twenty-five day old plants of Vicia faba were sprayed with different concentrations of two of the pesticides. The solutions were sprayed evenly on the plants by means of an atomizer at the rate of 2 ml. per plant per pot. Three pots were used for each treatment. Spraying was carried out in a greenhouse at 25^{o}C and the pots were returned to a cold frame 12 hours later. The above procedure was repeated after five days, so that each plant was sprayed twice before flowering.

7. Study of the pollen mother cells

Flower buds of treated Vicia faba plants were fixed in fresh Carnoy's fixative for 24 hours, and were stained by the Feulgen technique. Squashed were made in 45% acetic acid.

8. Microscopic observations

Slides were examined under light and phase contrast microscopy. The aim was to observe at least 300 dividing cells for each treatment. All the cells with chromosomal aberrations were noted. Selected cells were photographed under different magnifications using a Zeiss photo-microscope.

RESULTS

I. Morphological observations

1. Root growth in Tradescantia: Low concentrations (200 p.p.m. and 400 p.p.m.) of EMS for treatment periods of 3, 6 and 12 hours had no effect on root growth of Tradescantia, but a 12 hr. treatment with 600 p.p.m. showed a marked effect. Root growth was retarded and the zone of elongation was stunted in comparison with untreated roots.

Phosdrin and Bladex produced no obvious effects on root growth, irrespective of the concentration and treatment time.

Plants treated with Panogen 15 at dosages of 200, 400 and 600 p.p.m. for 3, 6, and 12 hours showed very poor root growth. Even after the concentration was decreased to 1.0, 2.0, and 5.0 p.p.m., and the treatment time shortened to 1, 2, and 3 hours, Panogen 15 still produced some inhibitory effects. In some cases the roots were bent in a hook-like fashion. This might not represent a complete inactivation, however, there was a deviation from the normal longitudinal polarity of mitosis. In addition, some of the root tips turned brown.

2. Toxic effects in Vicia faba: EMS produced no obvious morphological abnormality in roots of Vicia faba at the lower concentrations and shorter durations of treatment. But at higher concentrations and longer periods of treatment root growth was retarded.

Phosdrin produced some light brown spots on the leaves of Vicia faba after root tip treatment, but all the treated plants survived.

Bladex was found to be quite toxic to Vicia faba with the concentrations and time periods used. A few days after the root tips were treated, the leaves turned dark brown and ultimately all the plants died.

The higher concentrations and the longer periods of treatment with Panogen 15 were quite toxic to the whole root system of Vicia faba. The root tips bent upward making each root resemble a hook. For this reason it was necessary to reduce the concentrations to 1.0 p.p.m., 2.0 p.p.m. and 5.0 p.p.m., and the duration of treatment to 1, 2 and 3 hours.

Spraying, with concentrations of 200, 400 and 600 p.p.m. of Phosdrin, produced a few brown spots on the leaf margins of Vicia faba. Spraying with the same concentrations of Bladex was found to be extremely toxic for the plants. All the plants turned black and died.

II. Cytological observations

1. Chromosomal aberrations in root tip cells of Tradescantia

Tradescantia has 12 large chromosomes as shown in Plate I, Figure I. The data on chromosome aberrations induced by EMS and the three pesticides in the root tip cells of Tradescantia are presented in Table I, II, III and IV.

i) EMS: There was an increase in the percentage of chromosomal aberrations with increasing EMS concentration. Increasing treatment time produced no consistent trend in the percentage of abnormal cells

TABLE I. Frequency and distribution of chromosomal aberrations induced by ethyl methane sulfonate in Tradescantia root tips

Chemical	Treatment time	Concentration (p.p.m.)	Metaphase fragments	Anaphase fragments	Telophase fragments	Multipolar anaphases	Bridges	Bridges and fragments	Others[†]	Total no. of cells examined	Total no. of abnormal cells	% of abnormal cells	Mean % for each time period
EMS	3 hr	200	0	4	2	7	0	0	2	437	15	3.43*	
		400	3	8	5	0	0	0	1	300	17	5.67***	
		600	3	2	3	2	0	0	2	264	12	4.55**	4.55
	6 hr	200	3	4	0	1	0	0	2	325	10	3.08*	
		400	5	6	0	2	0	0	0	254	13	5.12**	
		600	1	1	2	2	7	0	0	258	13	5.04**	4.41
	12 hr	200	0	3	0	1	3	0	0	320	7	2.19**	
		400	5	3	0	1	0	0	3	305	12	3.93***	
		600	2	0	2	0	11	0	1	284	16	5.63***	3.92
Control			0	0	0	1	0	0	1	300	2	0.66	

[†] Includes lagging chromosomes, micronuclei, and broken bridges.

* Comparison of each treatment's data with the control data, using individual 2 X 2 contingency χ^2 tests, showed the treatment deviated significantly from control at the 0.05 level of probability.

** Significant at 0.01 level of probability.

*** Significant at 0.001 level of probability.

Figure 1. Chromosomal aberrations induced by EMS in *Tradescantia* and *Vicia faba* with 3 hour treatment period.

Figure 2. Chromosomal aberrations induced by EMS in *Tradescantia* and *Vicia faba* with 6 hour treatment period.

Figure 3. Chromosomal aberrations induced by EMS in *Tradescantia* and *Vicia faba* with 12 hour treatment period.

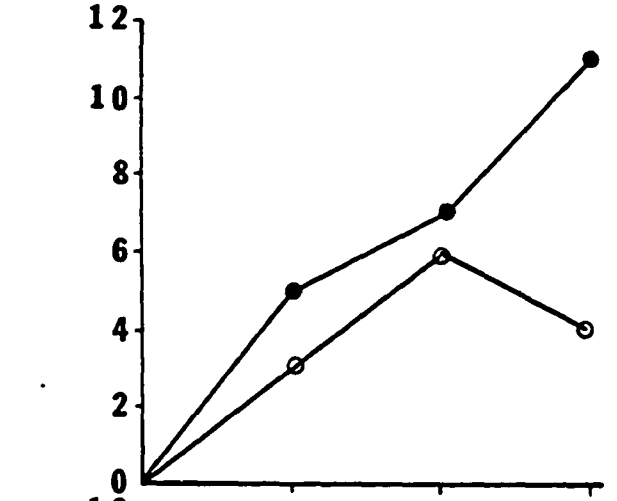

Fig.1

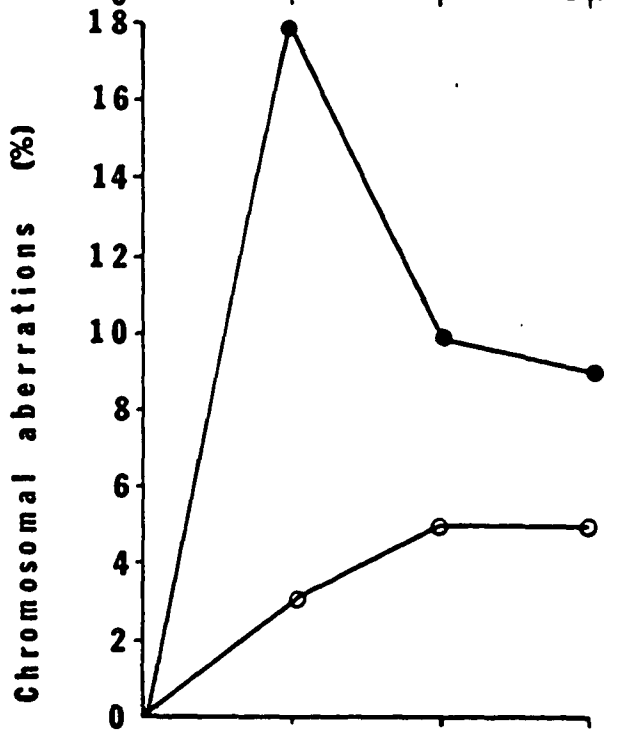

Fig.2

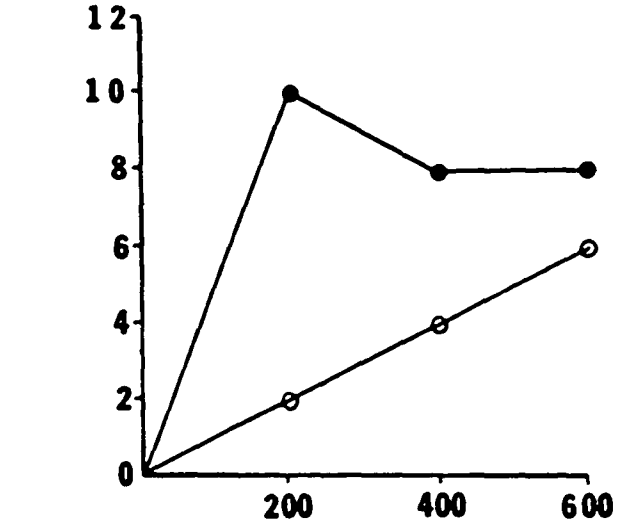

Fig.3

Figure 4a. Total frequency of different types of chromosomal aberrations induced by EMS in Tradescantia root tips.

Figure 4b. Total frequency of different types of chromosomal aberrations induced by EMS in Vicia faba root tips.

Abbreviations:

Mf = Metaphase fragments

Af = Anaphase fragments

Tf = Telophase fragments

Ma = Multipolar anaphases

Br = Bridges

Brf = Bridges with fragments

Mis = Miscellaneous

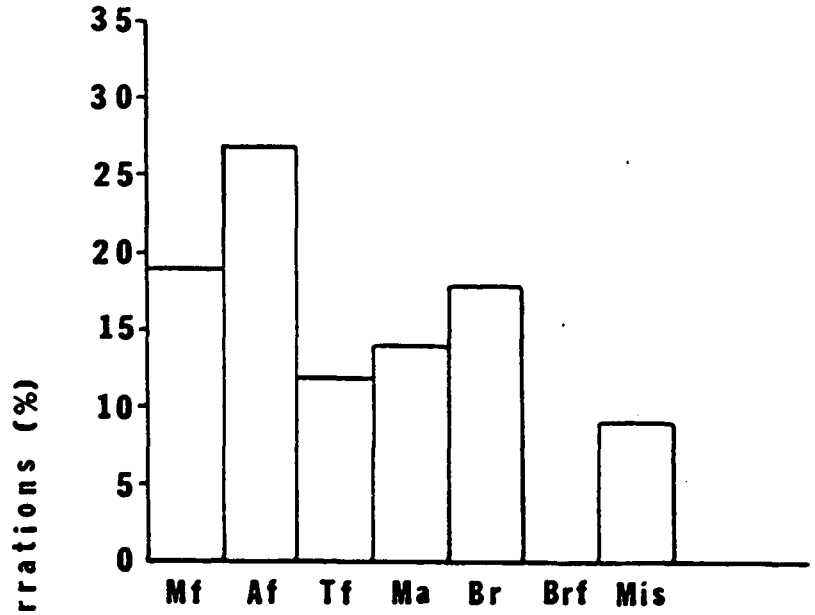
35
30
25
20
15
10
5
0
Mf
Af
Tf
Ma
Br
Brf
Mis

Fig.4a

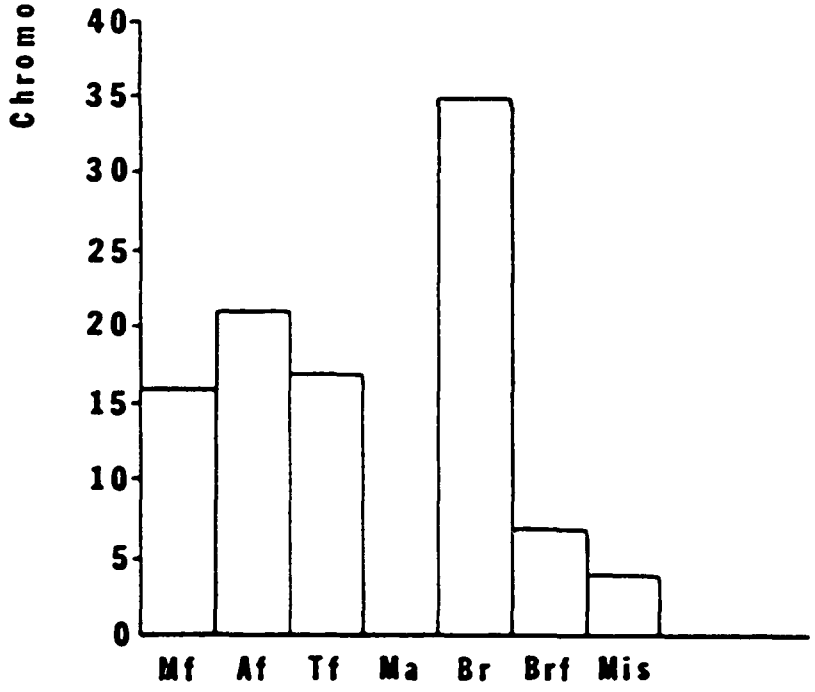
40
35
30
25
20
15
10
5
0
Mf
Af
Tf
Ma
Br
Brf
Mis
Chromosomal aberrations (%)
Types of aberrations

Fig.4b

(see Figures 1, 2 and 3). The most common types of chromosome aberrations observed were fragments and bridges. Multipolar anaphases and laggards were occasionally observed (see Figure 4a).

ii) Phosdrin: It can be seen from Table II and Figures 5, 6 and 7 that an increase in the concentration of this insecticide produced an increased frequency of chromosomal aberrations up to 6 hours of treatment. When the treatment time was increased to 12 hours, the trend was reversed. Fragments, and multipolar anaphases were the predominant chromosomal aberrations observed (see Figure 8a). Bridges, bridges with fragments, micronuclei, and laggards were also observed (see Plate I, Figures II-V).

iii) Bladex: An increase in the duration of treatment seemed to decrease the percentage of aberrations produced (see Table III). No correlation was found between the concentration of the herbicide and the percentage of aberrations produced (see Figures 9-11). Metaphase and anaphase fragments, multipolar anaphases, bridges, and other types of chromosomal aberrations were observed (see Figure 12a and Plate II, Figures VI and VII).

iv) Panogen 15: This fungicide, unlike Phosdrin and Bladex which act as radiomimetic agents, is a highly potent c-mitotic agent. The predominant, cytologically observable effect of Panogen 15, is c-mitotic action (see Plate II, Figures VIII-X). Much of the abnormal behavior of the chromosomes can be traced back to this action.

The effect of Panogen 15 resembles that of colchicine with two exceptions: (1) it is considerably more toxic than colchicine, and

TABLE II. Frequency and distribution of chromosomal aberrations induced by Phosdrin in Tradescantia root tips

Insecticide	Treatment time	Concentration (p.p.m.)	Metaphase fragments	Anaphase fragments	Telophase fragments	Multipolar anaphases	Bridges	Bridges and fragments	Others†	Total no. of cells examined	Total no. of abnormal cells	% of abnormal cells	Mean % for each time period
Phosdrin	3 hr	200	1	1	0	0	0	0	0	340	2	0.58	
		400	0	0	1	2	0	1	0	235	4	1.70*	
		600	0	4	0	4	2	0	1	311	11	3.53*	1.94
	6 hr	200	0	2	3	1	0	0	1	251	7	2.78*	
		400	1	1	0	0	2	0	1	156	5	3.20**	
		600	0	1	0	2	0	0	2	93	5	5.37**	3.78
	12 hr	200	0	1	0	7	0	0	2	304	10	3.28*	
		400	1	1	0	2	0	0	0	370	4	1.08	
		600	0	1	1	1	0	0	1	260	4	1.53	1.96
Control			0	0	0	1	0	0	1	323	2	0.62	

† Includes lagging chromosomes, micronuclei, and broken bridges.

* Comparison of each treatment's data with the control data, using individual 2 X 2 contingency χ^2 tests, showed the treatment deviated significantly from control at the 0.05 level of probability.

** Significant at 0.01 level of probability.

Figure 5. Chromosomal aberrations induced by Phosdrin in *Tradescantia* and *Vicia faba* with 3 hour treatment period.

Figure 6. Chromosomal aberrations induced by Phosdrin in *Tradescantia* and *Vicia faba* with 6 hour treatment period.

Figure 7. Chromosomal aberrations induced by Phosdrin in *Tradescantia* and *Vicia faba* with 12 hour treatment period.

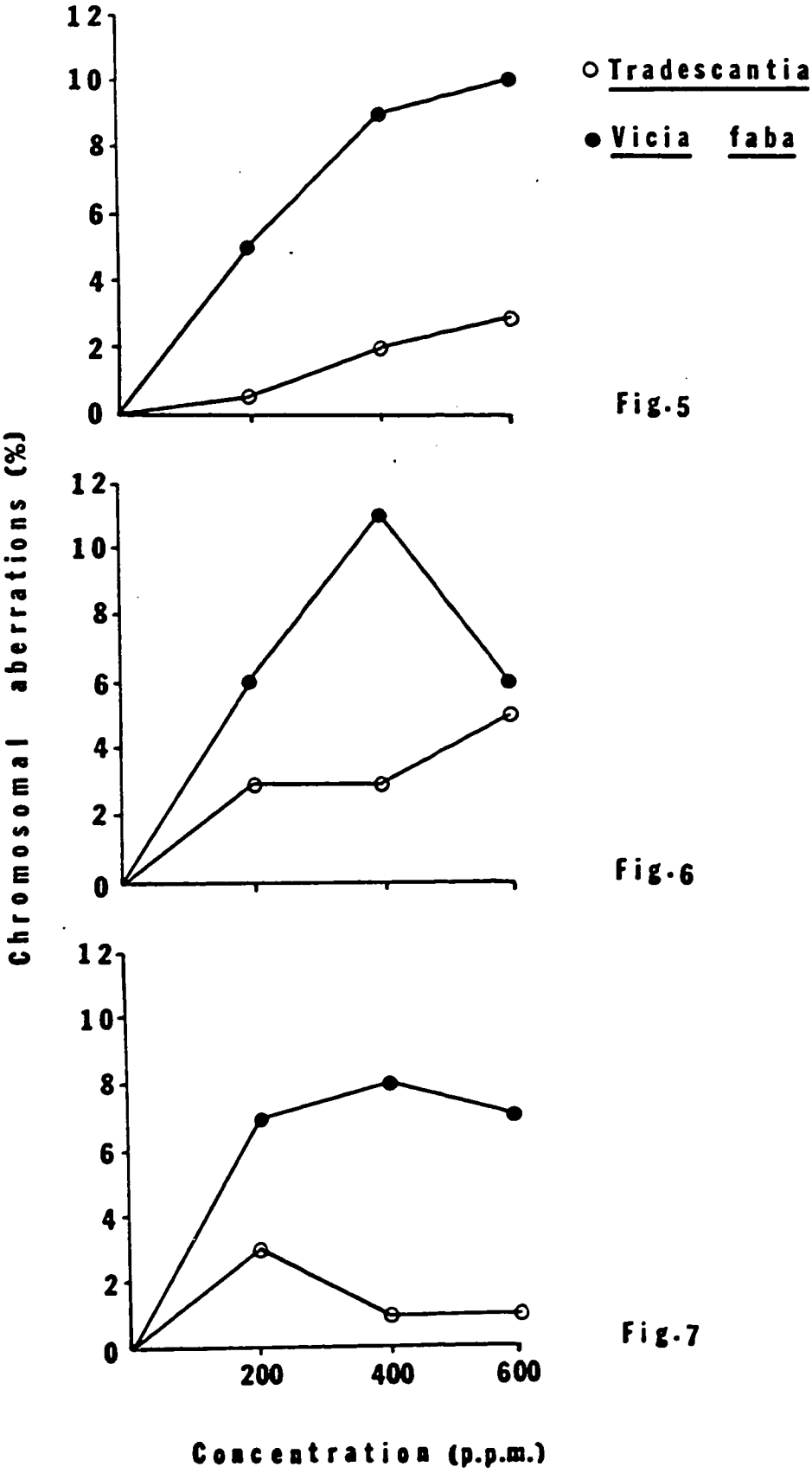
12
10
8
6
4
2
0
Tradescantia
Vicia faba
Fig.5
Chromosomal aberrations (%)
12
10
8
6
4
2
0
Fig.6
12
10
8
6
4
2
0
200
400
600
Fig.7
Concentration (p.p.m.)

Figure 8a. Total frequency of different types of chromosomal aberrations induced by Phosdrin in Tradescantia root tips.

Figure 8b. Total frequency of different types of chromosomal aberrations induced by Phosdrin in Vicia faba root tips.

Abbreviations:

Mf = Metaphase fragments

Af = Anaphase fragments

Tf = Telophase fragments

Ma = Multipolar anaphases

Br = Bridges

Brf = Bridges with fragments

Mis = Miscellaneous

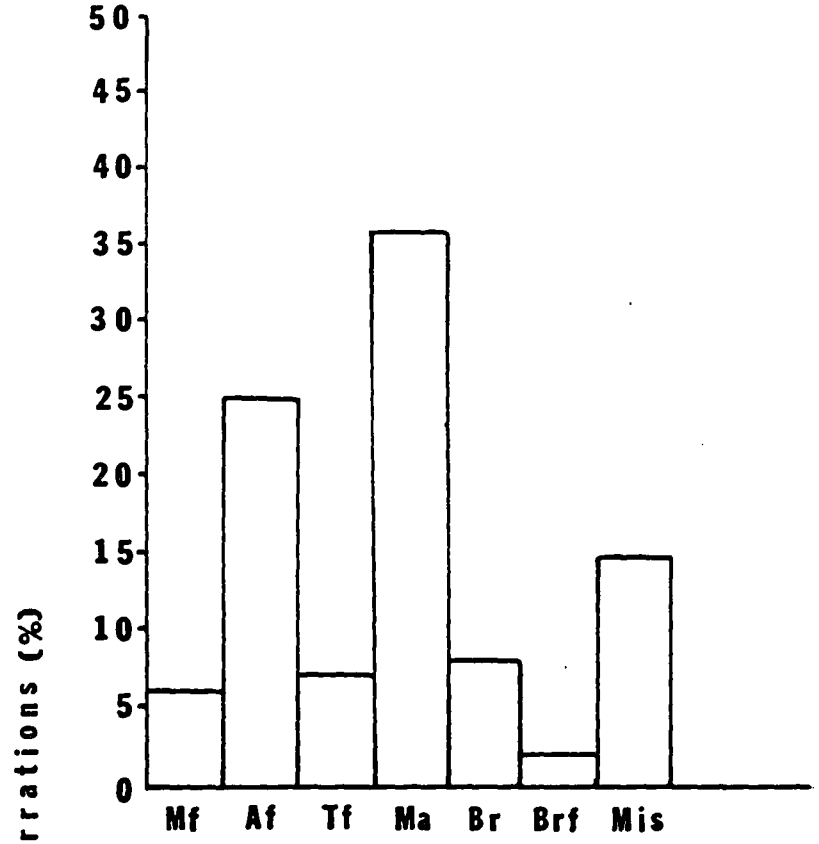
50
45
40
35
30
25
20
15
10
5
0
Mf
Af
Tf
Ma
Br
Brf
Mis

Fig.8a

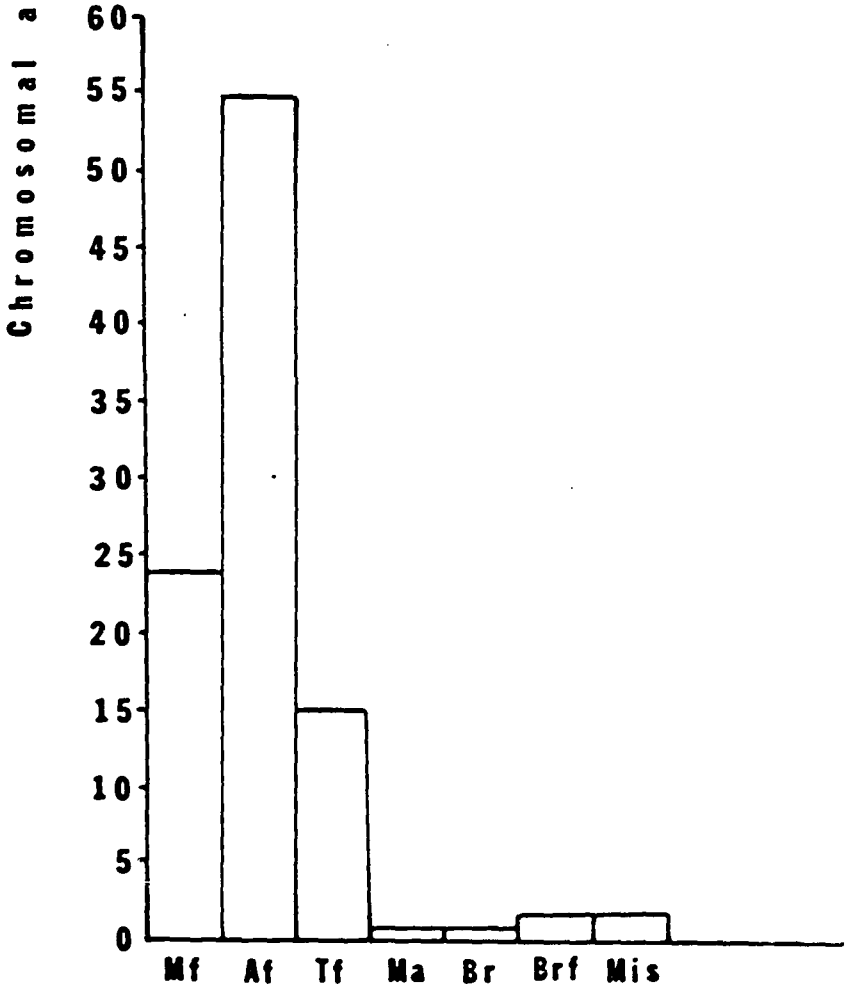
Chromosomal aberrations (%)
60
55
50
45
40
35
30
25
20
15
10
5
0
Mf
Af
Tf
Ma
Br
Brf
Mis
Types of aberrations

Fig.8b

Plate I

Figure I. A normal metaphase cell showing somatic chromosomes from root tip cells of Tradescantia (control). X ca. 600.

Figure II. A tripolar anaphase cell from root tips of Tradescantia treated with a concentration of 600 p.p.m. Phosdrin for a period of 3 hours. X ca. 575.

Figure III. An anaphase fragment in a root tip cell of Tradescantia after treatment with 200 p.p.m. Phosdrin for 3 hours. X ca. 625.

Figure IV. A telophase fragment in a root tip cell of Tradescantia after treatment with 600 p.p.m. Phosdrin for 12 hours. X ca. 1125.

Figure V. A prophase cell with a micronucleus in a root tip cell of Tradescantia after treatment with 200 p.p.m. Phosdrin for 12 hours. X ca. 1333.

Figure I

Figure II

Figure III

Figure IV

Figure V

TABLE III. Frequency and distribution of chromosomal aberrations induced by Bladex in Tradescantia root tips

Herbicide	Treatment time	Concentration (p.p.m.)	Metaphase fragments	Anaphase fragments	Telophase fragments	Multipolar anaphases	Bridges	Bridges and fragments	Others[†]	Total no. of cells examined	Total no. of abnormal cells	% of abnormal cells	Mean % for each time period
Bladex	3 hr	200	1	0	1	5	0	0	5	234	12	5.12**	
		400	0	2	0	1	1	0	0	214	4	1.86	
		600	1	0	0	1	1	0	7	127	10	7.87***	4.95
	6 hr	200	3	2	0	2	0	1	2	336	10	2.97*	
		400	0	0	0	4	1	0	0	322	5	1.55	
		600	0	4	0	4	0	0	1	194	9	4.63**	3.05
	12 hr	200	2	2	0	7	0	0	0	352	11	3.12*	
		400	1	1	0	1	0	0	1	210	4	1.90	
		600	2	1	0	3	0	0	0	268	6	2.23	2.42
Control			0	2	0	1	0	0	0	353	3	0.85	

[†] Includes lagging chromosomes, micronuclei, and broken bridges.

* Comparison of each treatment's data with the control data using individual 2 X 2 contingency χ^2 tests, showed the treatment deviated significantly from control at the 0.05 level of probability.

** Significant at 0.01 level of probability.

*** Significant at 0.001 level of probability.

Figure 9. Chromosomal aberrations induced by Bladex in *Tradescantia* and *Vicia faba* with 3 hour treatment period.

Figure 10. Chromosomal aberrations induced by Bladex in *Tradescantia* and *Vicia faba* with 6 hour treatment period.

Figure 11. Chromosomal aberrations induced by Bladex in *Tradescantia* and *Vicia faba* with 12 hour treatment period.

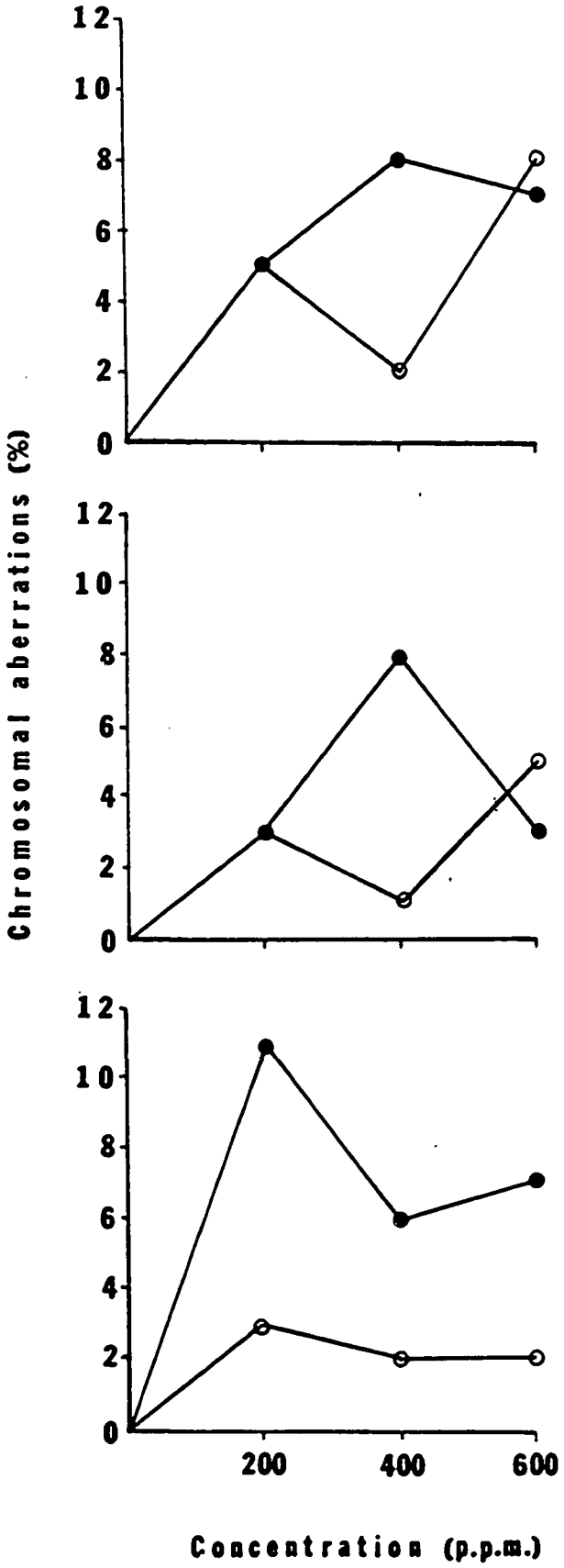

Fig.9

Fig.10

Fig.11

Figure 12a. Total frequency of different types of chromosomal aberrations induced by Bladex in Tradescantia root tips.

Figure 12b. Total frequency of different types of chromosomal aberrations induced by Bladex in Vicia faba root tips.

Abbreviations:

Mf = Metaphase fragments

Af = Anaphase fragments

Tf = Telophase fragments

Ma = Multipolar anaphases

Br = Bridges

Brf = Bridges with fragments

Mis = Miscellaneous

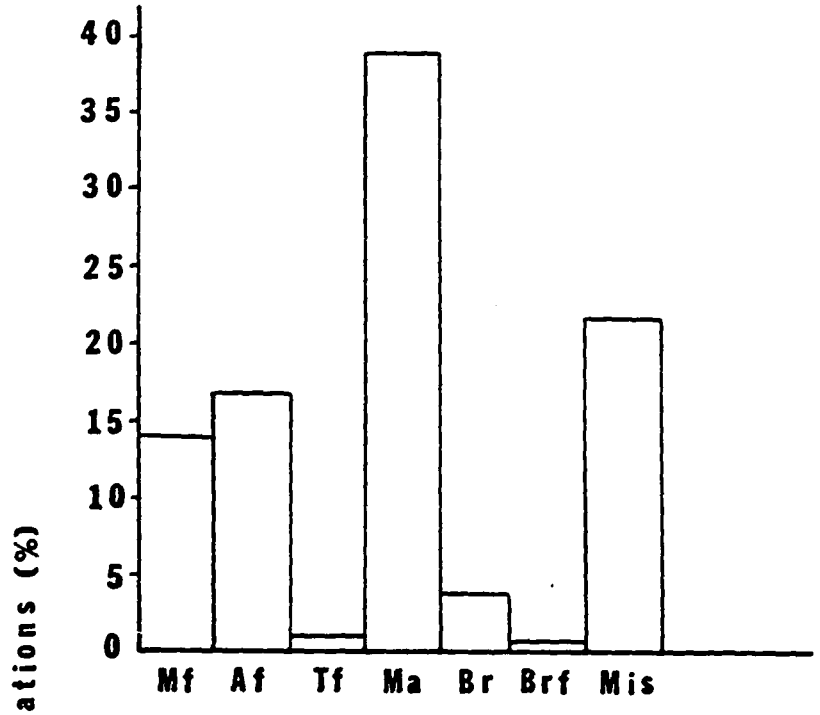

Fig.12a

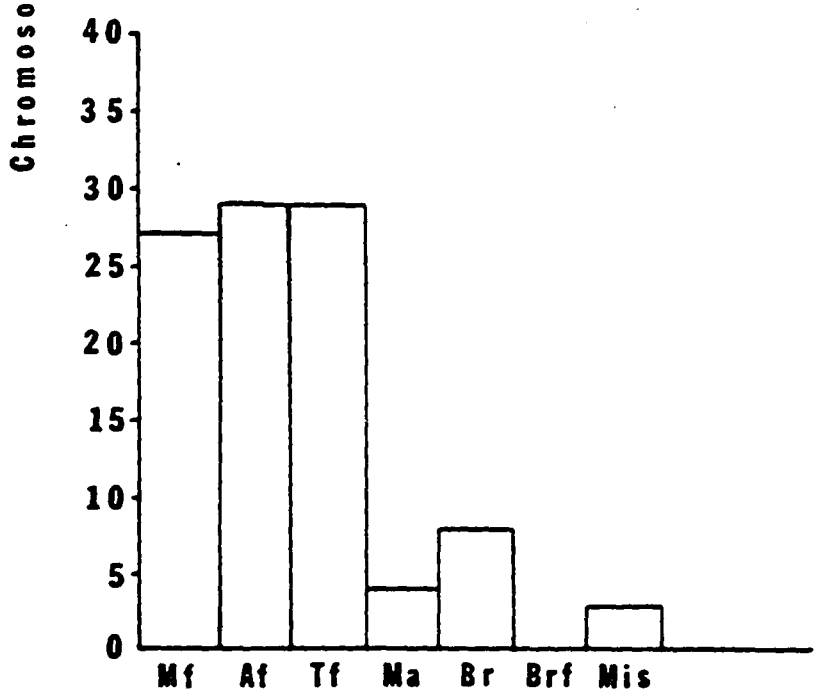

Fig.12b

TABLE IV. Percentage of c-mitoses induced by Panogen 15 in Tradescantia root tips

Fungicide	Treatment time	Concentration (p.p.m.)	No. of cells examined	Normal cells	c-mitoses	Polyploid cells	Strong toxicity	% of c-mitoses	Mean % for each time period
Panogen 15	1 hr	1	348	1	329	2	16	95.11***	
		2	265	0	210	0	55	79.24***	
		5	284	0	7	0	277	2.46	58.94
	2 hr	1	324	0	324	0	0	100.00***	
		2	306	0	22	0	284	7.18**	
		5	251	0	5	0	246	1.99	36.39
	3 hr	1	465	2	411	1	49	88.60***	
		2	379	0	343	0	36	90.50***	
		5	300	0	156	0	144	52.00***	77.033
Colchicine	1 hr	1000	311	0	307	0	4	98.71***	
Control			346	337	9	0	0	2.60	

** Comparison of each treatment's data with the control data using contingency χ^2 tests showed the treatment deviated significantly from control at the 0.01 level of probability.

*** Significant at 0.001 level of probability.

Figure 13. C-mitoses induced by Panogen 15 in Tradescantia and Vicia faba with 1 hour treatment period.

Figure 14. C-mitoses induced by Panogen 15 in Tradescantia and Vicia faba with 2 hour treatment period.

Figure 15. C-mitoses induced by Panogen 15 in Tradescantia and Vicia faba with 3 hour treatment period.

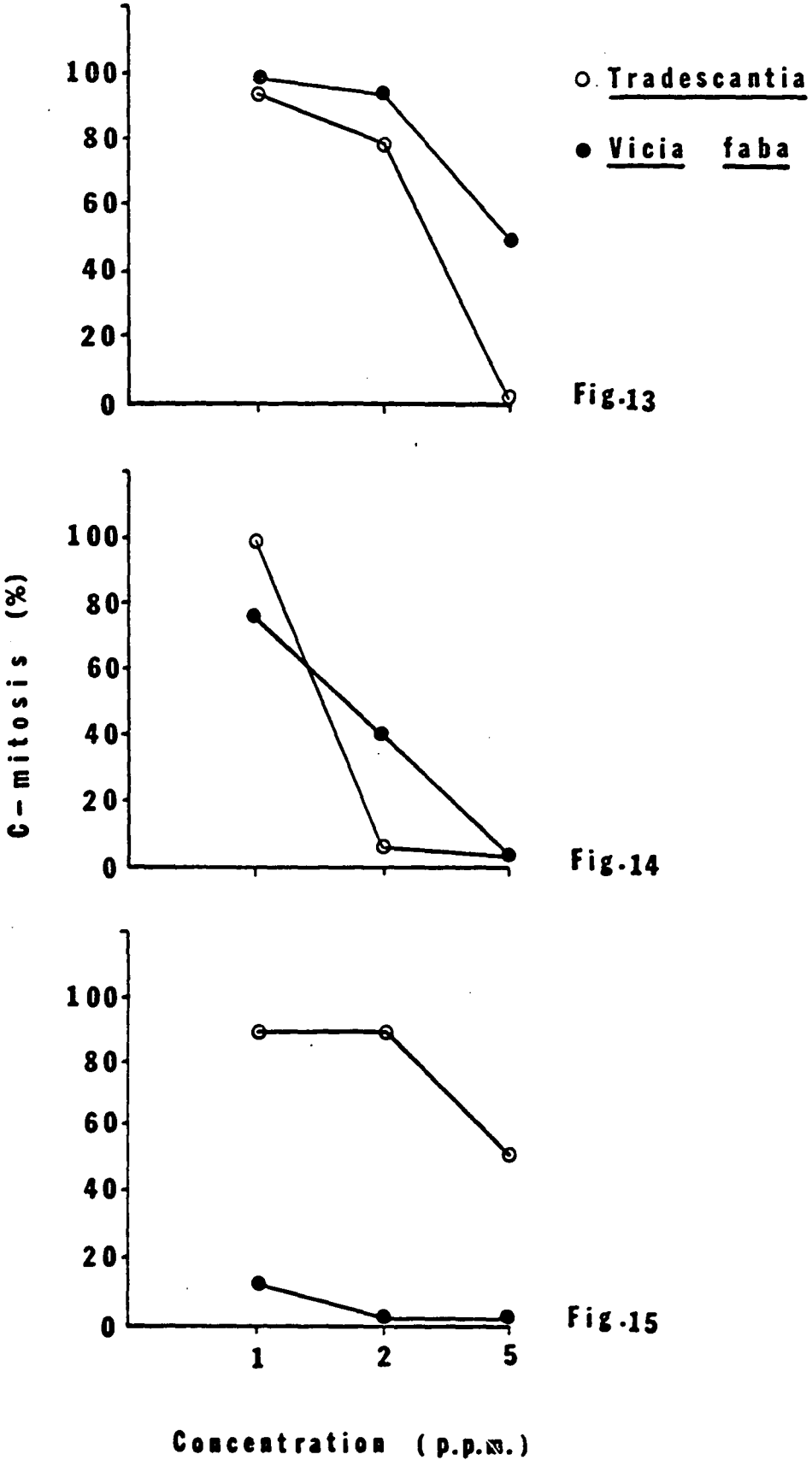

Fig.13

Fig.14

Fig.15

Plate II

Figure VI. An anaphase cell with a lagging chromosome from root tips of Tradescantia treated with 200 p.p.m. Bladex for 6 hours. X ca. 833.

Figure VII. An anaphase cell with multiple fragments from root tips of Tradescantia treated with 200 p.p.m. Bladex for 6 hours. X ca. 783.

Figure VIII. A c-metaphase cell from root tips of Tradescantia treated with 1 p.p.m. Panogen 15 for 2 hours. X ca. 600.

Figure IX. A part of Figure VIII magnified showing transverse interconnections between two chromatids. X ca. 2000.

Figure X. A multinucleate cell from root tips of Tradescantia treated with 1 p.p.m. Panogen 15 for 1 hour. X ca. 496.

Figure XI. Cells of Tradescantia root tips showing strong toxicity after treatment with 5 p.p.m. Panogen 15 for 2 hours. X ca. 470.

Figure VI

Figure VII

Figure VIII

Figure IX

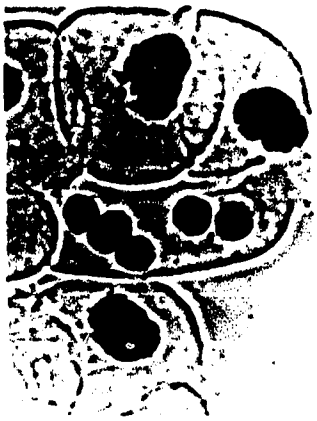

Figure X

Figure XI

(2) the dose range giving rise to complete c-mitosis is much narrower than in the case of colchicine (see Table IV). Concentrations as low as 10 and 15 p.p.m. caused extensive cell death. Consequently, the concentrations were reduced to 1 p.p.m., 2 p.p.m. and 5 p.p.m.

Since a long treatment time also had a toxic effect on the cells, the treatment times were reduced to 1, 2 and 3 hours. There was a gradual decrease in the percentage of c-mitoses as the concentration was increased from 1 p.p.m. to 5 p.p.m. due to increased lethality (see Figures 13 to 16).

Cells considered toxic had irregular, lobate chromatin masses, as shown in Plate II, Figure XI, and there was a tendency for the cytoplasm to stain more deeply. If the treatment time was short (2 hrs.), c-mitoses predominated. When the root tips were treated with 1 p.p.m. for 2 hours, 100% c-mitoses were observed (see Table IV). Panogen 15 treatment was often extremely favorable for obtaining a great number of well spread metaphases with unusually clear morphological details of the chromosomes. Contraction was variable, but sometimes ideal for morphological analysis. A difference in the stainability of chromosomes was also observed: the contour of the chromosomes was darkly stained in comparison with the inner zone which was much more lightly stained. The most interesting phenomenon observed from Panogen 15 treatment was the presence of conspicuous transverse interconnections between the chromatids (see Plate II, Figures VIII and IX). Polyploid and multinucleate cells (see Plate II, Figure X) were also observed but no chromosome fragments.

2. Chromosomal aberrations in root tip cells of Vicia faba

The data on the chromosome aberrations produced by EMS, Phosdrin, Bladex and Panogen 15 are given in Tables V, VI, VII and VIII.

i) EMS: With a 3 hour treatment period, increasing concentrations of EMS produced an increase in the number of aberrations (see Figure 1); but after 6 and 12 hours, no such trend was observed (see Figures 2 and 3). With a 12 hour treatment period the cells were shrunken in comparison to control cells. Of the chromosome abnormalities, telophase bridges were most frequent, followed by fragments and lagging chromosomes (see Figure 4b and Plate III, Figure XII).

ii) Phosdrin: The types of chromosomal aberrations produced by Phosdrin parallelled those produced in Tradescantia with this insecticide. However, the frequency of anaphase fragments was higher in Vicia faba (see Figure 9b). It is interesting to note that a 400 p.p.m. treatment, on the average, produced a higher percentage of abnormal cells than did the other two concentrations (200 p.p.m. and 600 p.p.m.) (see Table VI, and Figures 5-7). Some of the representative cells with typical chromosome abnormalities are shown in Plates III and IV, Figures XIII-XX.

iii) Bladex: This herbicide, like Phosdrin, produced the highest average percentage chromosomal aberrations at 400 p.p.m. in Vicia faba (see Figures 9-11). Fragments were more frequent than any other type of chromosome aberration (see Figure 12b and Plate IV, Figures XXI-XXIII).

TABLE V. Frequency and distribution of chromosomal aberrations induced by ethyl methane sulfonate in Vicia faba root tips

Chemical	Treatment time	Concentration (p.p.m.)	Metaphase fragments	Anaphase fragments	Telophase fragments	Multipolar anaphases	Bridges	Bridges and fragments	Others†	Total no. of cells examined	Total no. of abnormal cells	% of abnormal cells	Mean % for each time period
EMS	3 hr	200	0	10	11	0	0	0	0	388	21	5.41***	
		400	9	8	7	0	0	0	1	331	25	7.55***	
		600	17	0	0	0	0	16	2	303	35	11.55***	8.17
	6 hr	200	10	39	8	0	0	0	0	315	57	18.10***	
		400	7	0	0	0	24	5	2	360	38	10.55***	
		600	2	8	19	0	1	0	2	340	32	9.41***	12.69
	12 hr	200	1	0	0	0	39	0	0	382	40	10.47***	
		400	0	0	0	0	31	0	0	378	31	8.20***	
		600	3	0	3	1	12	1	6	334	26	7.78***	8.82
Control			0	4	1	0	0	0	0	431	5	1.16	

† Includes lagging chromosomes, micronuclei, and broken bridges.

*** Comparison of each treatment's data with the control data using individual 2 X 2 contingency χ^2 tests, showed the treatment deviated significantly from control at the 0.001 level of probability.

TABLE VI. Frequency and distribution of chromosomal aberrations induced by Phosdrin in Vicia faba root tips

Insecticide	Treatment time	Concentration (p.p.m.)	Metaphase fragments	Anaphase fragments	Telophase fragments	Multipolar anaphases	Bridges	Bridges and fragments	Others[†]	Total no. of cells examined	Total no. of abnormal cells	% of abnormal cells	Mean % for each time period
Phosdrin	3 hr	200	5	10	0	1	1	0	0	319	17	5.33***	
		400	16	7	0	0	1	3	4	348	31	8.91***	
		600	5	17	10	0	0	0	0	303	32	10.56***	8.27
	6 hr	200	0	12	5	0	0	1	0	314	18	5.73***	
		400	16	13	7	0	0	0	0	320	36	11.25***	
		600	11	7	1	0	0	0	0	317	19	5.99***	7.66
	12 hr	200	4	17	5	0	0	1	0	378	27	7.14***	
		400	1	23	6	0	0	0	0	358	30	8.37***	
		600	0	24	2	1	1	0	0	374	28	7.48***	7.66
Control			0	3	0	0	0	0	0	398	3	0.75	

[†] Includes lagging chromosomes, micronuclei, and broken bridges.

*** Comparison of each treatment's data with the control data using individual 2 X 2 contingency χ^2 tests showed the treatment deviated significantly from control at the 0.001 level of probability.

TABLE VII. Frequency and distribution of chromosomal aberrations induced by Bladex in Vicia faba root tips

Herbicide	Treatment time	Concentration (p.p.m.)	Metaphase fragments	Anaphase fragments	Telophase fragments	Multipolar anaphases	Bridges	Bridges and fragments	Others†	Total no. of cells examined	Total no. of abnormal cells	% of abnormal cells	Mean % for each time period
Bladex	3 hr	200	1	10	4	1	0	0	0	310	16	5.16**	
		400	15	7	1	1	0	0	0	304	24	7.89***	
		600	3	16	3	3	0	0	0	366	25	6.83***	6.62
	6 hr	200	4	1	3	0	0	0	1	255	9	3.53*	
		400	10	6	15	0	0	0	0	365	31	8.49***	
		600	4	0	3	0	0	0	0	229	7	3.05	5.02
	12 hr	200	4	2	6	2	9	0	4	254	27	10.62***	
		400	8	2	3	0	6	0	0	325	19	5.84***	
		600	0	10	16	0	0	0	0	344	26	7.55***	8.00
Control			2	0	1	0	0	0	0	314	3	0.955	

† Includes lagging chromosomes, micronuclei, and broken bridges.

* Comparison of each treatment's data with the control data using individual 2 X 2 contingency χ^2 tests showed the treatment deviated significantly from control at the 0.05 level of probability.

** Significant at 0.01 level of probability.

*** Significant at 0.001 level of probability.

Plate III

Figure XII. An anaphase cell with a broken bridge and fragments from root tip cells of Vicia faba treated with 400 p.p.m. EMS for 6 hours. X ca. 1075.

Figure XIII. A metaphase cell with fragments from root tip cells of Vicia faba treated with 400 p.p.m. Phosdrin for 6 hours. X ca. 880.

Figure XIV. An anaphase cell with fragments from root tip cells of Vicia faba treated with 400 p.p.m. Phosdrin for 3 hours. X ca. 550.

Figure XV. An anaphase cell with fragments from root tip cells of Vicia faba treated with 600 p.p.m. Phosdrin for 3 hours. X ca. 733.

Figure XVI. An anaphase cell with multiple fragments from root tip cells of Vicia faba treated with 400 p.p.m. Phosdrin for 12 hours. X ca. 671.

Figure XVII. An anaphase cell with bridge and fragments from root tip cells of Vicia faba treated with 400 p.p.m. Phosdrin for 3 hours. X ca. 1600.

Figure XII

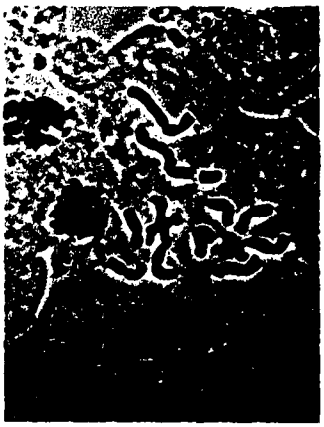

Figure XIII

Figure XIV

Figure XV

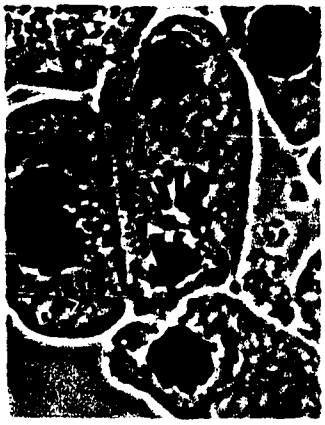

Figure XVI

Figure XVII

Plate IV

Figure XVIII. A telophase cell with fragments from root tip cells of Vicia faba treated with 600 p.p.m. Phosdrin for 3 hours. X ca. 860.

Figure XIX. A telophase cell with a bridge from root tip cells of Vicia faba treated with 400 p.p.m. Phosdrin for 3 hours. X ca. 2100.

Figure XX. A telophase cell with a broken bridge and a fragment from root tip cells of Vicia faba treated with 400 p.p.m. Phosdrin for 3 hours. X ca. 1466.

Figure XXI. A metaphase cell with a chromosome and a chromatid break from root tip cells of Vicia faba treated with 200 p.p.m. Bladex for 12 hours. X ca. 460.

Figure XXII. An anaphase cell with a lagging chromosome from root tip cells of Vicia faba treated with 200 p.p.m. Bladex for 6 hours. X ca. 1100.

Figure XXIII. A telophase cell with an incomplete bridge (gaps) from root tip cells of Vicia faba treated with 200 p.p.m. Bladex for 12 hours. X ca. 820.

Figure XVIII

Figure XIX

Figure XX

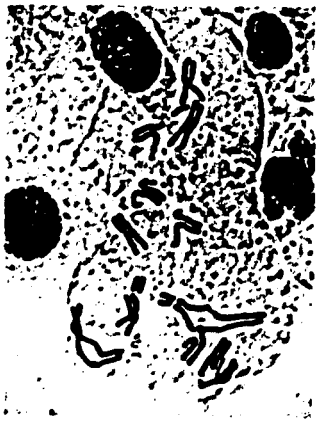

Figure XXI

Figure XXII

Figure XXIII

The percentage of chromosome abnormalities produced by EMS, Phosdrin and Bladex in Tradescantia and Vicia faba are presented in Figures 17a and 17b, respectively.

iv) Panogen 15: The observations on toxicity and c-mitosis produced by this mercury fungicide in Vicia faba are in good accordance with the results obtained in Tradescantia (see Figures 13-16). However, a treatment period of 3 hours was found to be more toxic to the cells of Vicia faba than to those of Tradescantia (see Figure 15). Illustrations of c-metaphase and multinucleate cells are provided in Plate V, Figures XXIV and XXV.

3. Chromosomal aberrations in pollen mother cells of Vicia faba

Phosdrin: The cytological results obtained from meiotic studies are summarized in Table IX. This insecticide produced a higher number of chromosomal aberrations in pollen mother cells than in root tip cells of Vicia faba (see Figure 18). Treatment with 400 p.p.m. of Phosdrin produced the highest number of chromosome abnormalities both in root tip cells and pollen mother cells. The majority of the abnormal cells were characterized by chromosome stickiness, nuclear clumping, chromosome fragments, bridges, and asynchronous and unequal separation of the chromosomes in anaphase II (see Plate V, Figures XXVI-XXIX).

Figure 16. C-mitoses induced by Panogen 15 in Tradescantia and Vicia faba (pooled data).

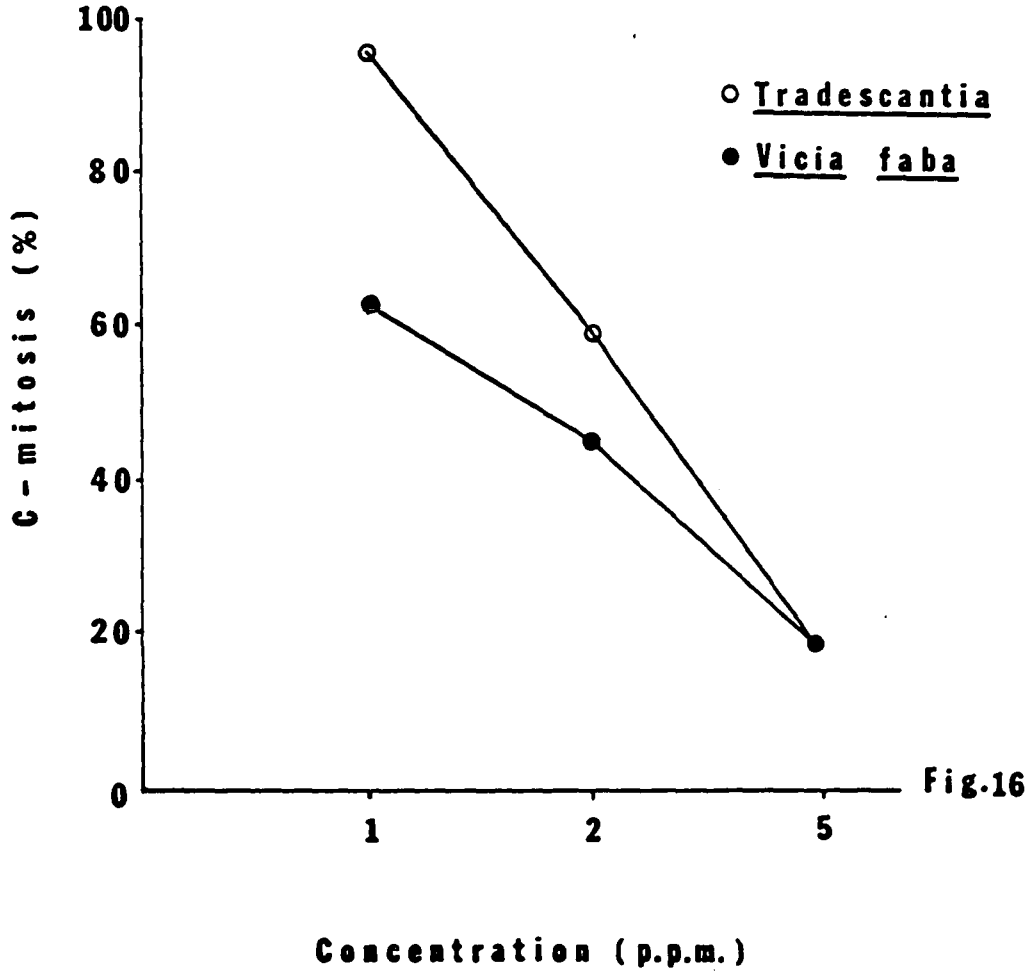
100
80
60
40
20
0
C-mitosis (%)
1
2
5
Concentration (p.p.m.)
Tradescantia
Vicia faba

Fig.16

Figure 17a. Total chromosomal aberrations induced by the pesticides in *Tradescantia* root tips (pooled data).

Figure 17b. Total chromosomal aberrations induced by the pesticides in *Vicia faba* root tips (pooled data)

Abbreviations:

EMS = Ethyl methane sulfonate

P = Phosdrin

B = Bladex

O = Control

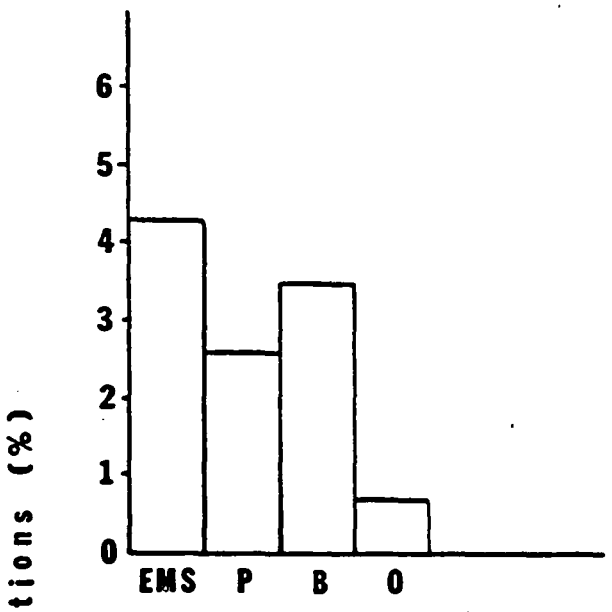

Fig.17a

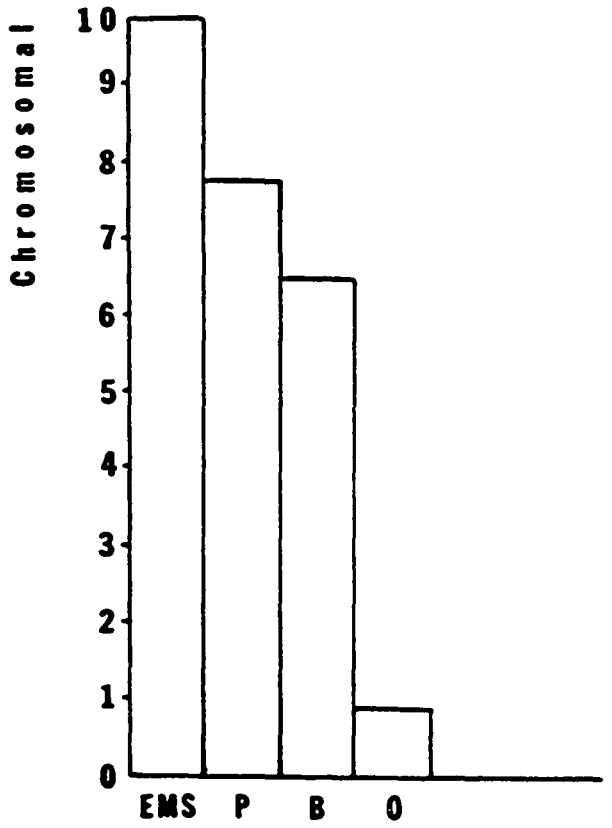

Fig.17b

TABLE VIII. Percentage of c-mitoses induced by Panogen 15 in Vicia faba root tips

Fungicide	Treatment time	Concentration (p.p.m.)	No. of cells examined	Normal cells	c-mitoses	Polyploid cells	Strong toxicity	% of c-mitoses	Mean % for each time period
Panogen 15	1 hr	1	333	5	328	0	0	98.50***	
		2	339	17	322	0	0	94.98***	
		5	333	0	164	3	166	50.15	81.21
	2 hr	1	368	0	270	10	88	76.08***	
		2	312	0	100	22	190	39.10***	
		5	370	0	13	0	357	3.51	39.56
	3 hr	1	263	1	33	0	229	12.55***	
		2	314	6	6	0	302	1.91*	
		5	272	0	8	0	264	2.94**	5.8
Colchicine	1 hr	1000	370	0	81	0	289	21.89	
Control			321	321	0	0	0	0	

* Comparison of each treatment's data with the control data using individual 2 X 2 contingency χ^2 tests showed the treatment deviated significantly from control at the 0.05 level of probability.
** Significant at 0.01 level of probability.
*** Significant at 0.001 level of probability.

TABLE IX. Frequency and types of chromosomal aberrations induced by Phosdrin in pollen mother cells of Vicia faba

Insecticide	No. of plants	Concentration (p.p.m.)	No. of cells examined	No. of abnormal cells†	% of abnormal cells
Phosdrin	3	200	419	41	9.79***
	3	400	402	136	33.83***
	3	600	406	75	18.47***
Control	3	0	349	1	0.286***

† Abnormalities included chromosome stickiness, nuclear clumping, chromosome fragments, chromosome bridges, and asynchronous and unequal division of chromosomes at Anaphase II.

*** Comparison of each treatment's data with the control data using individual 2 X 2 contingency χ^2 tests showed the treatment deviated significantly from control at the 0.001 level of probability.

Figure 18. Total chromosomal aberrations induced by Phosdrin in root tips and pollen mother cells of *Vicia faba*.

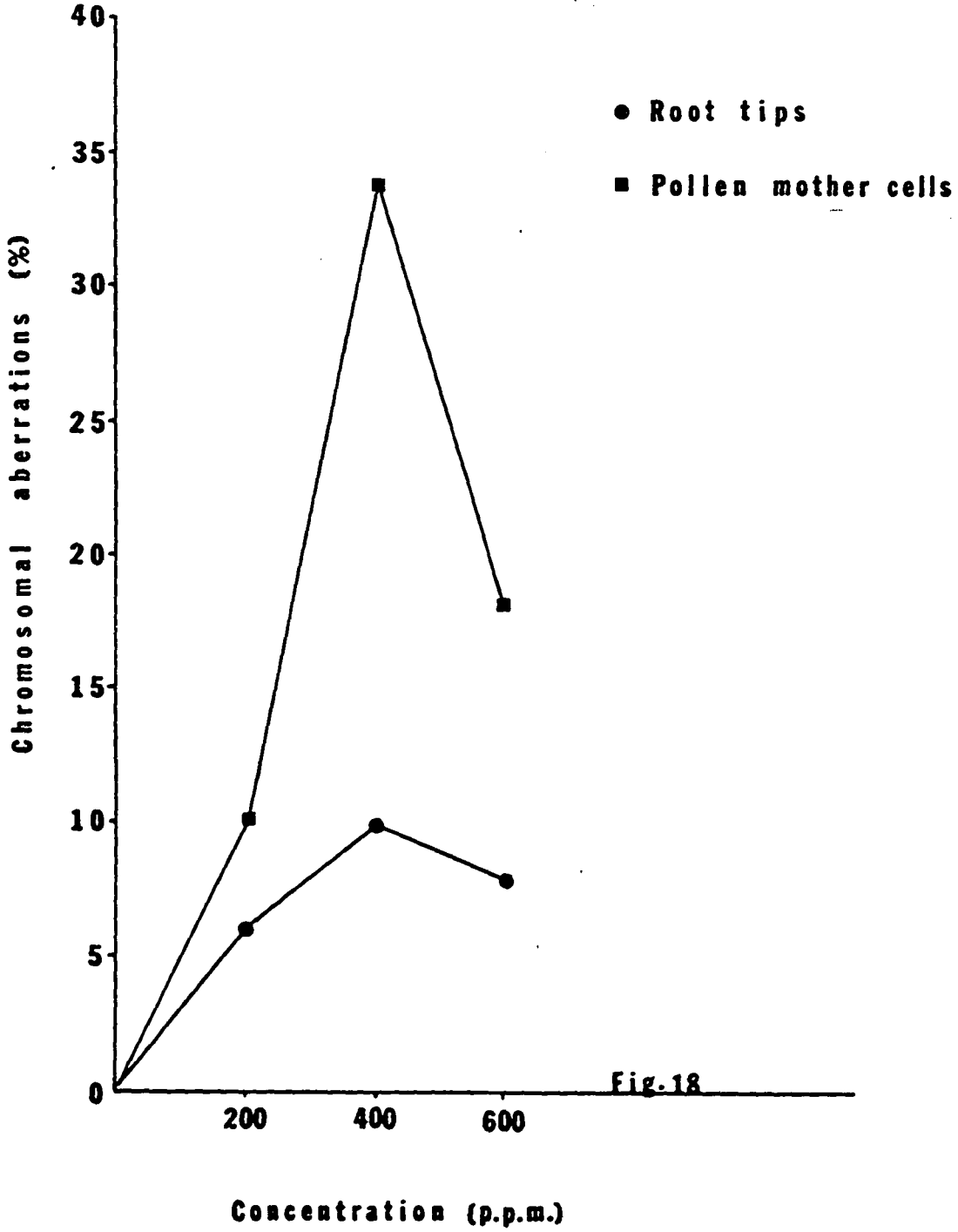
40
35
30
25
20
15
10
5
0
Chromosomal aberrations (%)
● Root tips
■ Pollen mother cells
200
400
600
Concentration (p.p.m.)

Fig.18

Plate V

Figure XXIV. A c-metaphase cell with chromosomes showing transverse striations from root tip cells of Vicia faba treated with 5 p.p.m. Panogen 15 for 1 hour. X ca. 1000.

Figure XXV. A multinucleate cell from root tips of Vicia faba treated with 5 p.p.m. Panogen 15 for 1 hour. X ca. 840.

Figure XXVI. A pollen mother cell showing a normal meiotic prophase in Vicia faba (control). X ca. 455.

Figure XXVII. A meiotic prophase in a pollen mother cell showing clumping of chromosomes in a Vicia faba plant sprayed with 600 p.p.m. Phosdrin. X ca. 427.

Figure XXVIII. Anaphase II with bridges in a Vicia faba plant sprayed with 400 p.p.m. Phosdrin. X ca. 1125.

Figure XXIX. Microspores of different sizes from anthers of a Vicia faba plant sprayed with 600 p.p.m. Phosdrin. X ca. 390.

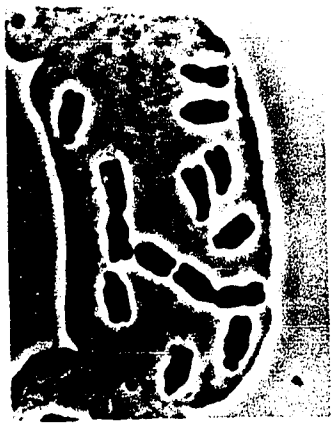

Figure XXIV

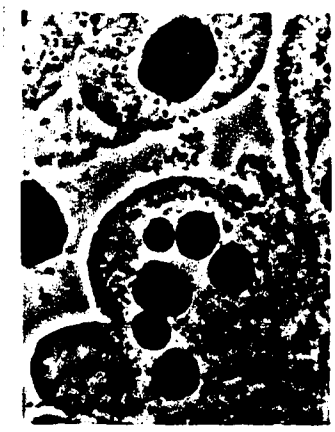

Figure XXV

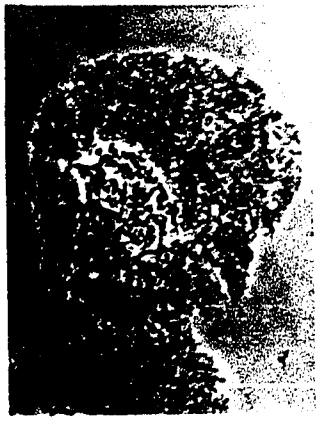

Figure XXVI

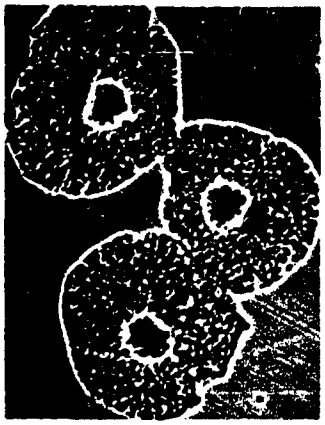

Figure XXVII

Figure XXVIII

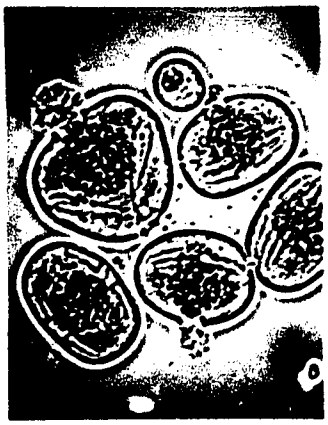

Figure XXIX

DISCUSSION

The insecticide Phosdrin and the herbicide Bladex, like the chemical mutagen EMS, are radiomimetic, that is they produce the same kind of effects as X-rays. The fungicide Panogen 15 is a potent c-mitotic agent and is comparable in its effects to the chemical action of colchicine.

The mode of action of most pesticides is not known. However, it has been shown that pesticides containing organophosphates have a radiomimetic effect in a number of plant species (Veleminsky and Gichner, 1963; Wuu and Grant, 1966a, 1967a, 1967b; Sax and Sax, 1968; Reddy and Rao, 1969). Phosdrin ($C_7H_{13}O_6P$) is an organophosphate compound containing one atom of phosphorus which probably accounts for the radiomimetic properties found in this study.

The mode of action of Bladex [2-(4-chloro-6-ethylamino-s-triazin-2-ylamino)-2-methyl-propionitrile] is also not known. It is a triazine herbicide. A number of triazine herbicides cause both mitotic and meiotic chromosomal aberrations in a variety of plant species (Wuu and Grant, 1966a, 1967a, 1967b; Liang *et al.*, 1967; Stroyev, 1968).

The types of chromosome aberrations induced by Phosdrin and Bladex are similar in *Tradescantia* and *Vicia faba*, however, the plants varied in their sensitivity to the pesticides. On the whole, the frequency of chromosomal aberrations produced by Phosdrin and Bladex in *Vicia faba* is higher than in *Tradescantia*. The most

common types of chromosome aberrations observed were metaphase, anaphase, and telophase fragments. Other abnormalities such as bridges, bridges with fragments, multipolar anaphases, laggards, micronuclei and stickiness of chromosomes were also observed. The molecular processes by which structural chromosome aberrations take place are as yet largely unknown. In all probability they will continue to be unknown until our knowledge of chromosome structure becomes more than speculation.

The effect of Panogen 15 resembles that of colchicine, except the former is more toxic than the latter. It is assumed that mercury compounds react with sulfhydryl groups (SH), so the possible role of sulfhydryl groups in the formation of the mitotic apparatus is of primary interest. Mazia (1955 and 1961) has proposed the probable mechanism of colchicine action. The synthesis of the mitotic apparatus is initiated by an increase of protein SH, resulting from a reduction of intramolecular S-S bonds. The soluble SH groups are involved in this process and therefore show a simultaneous decrease. The protein sulfhydryl groups thus formed are then oxidized to new disulfide bonds, but now between molecules giving rise to protein polymers. At the same time soluble SH is again released forming a gel. The final step in the formation of spindle fibres involves the transformation of this gel from a disordered to an ordered condition. The mechanism of this step may involve hydrogen bonds. The action of colchicine presumably occurs at this step.

Based on the above information, Ramel (1969) has suggested three possible ways in which mercury compounds could interfere with this system: (1) The mercury compounds may cause a c-mitotic effect through direct action on sulfhydryl groups of proteins actually involved in the formation of the spindle, preventing them from polymerization, (2) the mercury compounds may also react with disulfide groups already formed, (3) the mercury compounds may act in a more indirect way, through association with SH groups not immediately involved in the formation of the spindle, but essential for the initiation of the process in a catalytic way.

To test the reactions of mercurials with sulfhydryl groups, Ramel (1969) treated Allium roots with combinations of phenyl mercury hydroxide and 2,3-dimercapto-1-propanol (BAL). The SH group of BAL is known to react very strongly with mercury compounds and with other SH-reagents. If the action of the mercury compounds is caused by linkages with sulfhydryl groups of proteins, directly or indirectly involved in the synthesis of mitotic apparatus, BAL should act as an antagonist to the mercurials. From Ramel's results it can be seen that BAL does inhibit the c-mitotic action of the mercury compound which he used. This supports the hypothesis that the mitotic apparatus is formed by sulfhydryl-disulfide interactions and also supports the idea that mercury compounds interfere with the sulfhydryl reactions.

In the present study low concentrations (1,2 p.p.m.) of Panogen 15

(a mercury based fungicide) have been found to be extremely efficient in causing c-mitosis. Chromosomes were well spread and unusually clear. Interphase cells with lobated nuclei, micro- and macro-nuclei and multinuclei were often observed. Some of these abnormalities may have been formed as a result of the chromosomes separating to the poles in an erratic manner. From the c-mitotic action of Panogen 15, polyploid as well as aneuploid cells were also occasionally observed.

Besides causing a contraction of the chromosomes, Panogen 15 affected chromosome morphology in other ways. There was differential staining within each chromosome and a peculiar phenomenon was the presence of transverse interconnections between the chromatids. This was observed in both Tradescantia and Vicia faba treated with Panogen 15. Golomb and Bahr (1971), using a scanning electron microscopic technique, have shown the existence of fibre bridges between chromatid pairs in isolated human chromosomes. The transverse connections observed from Panogen 15 treatment appear to be comparable to the electron microscopic observations.

A comparison between the chromosomal abnormalities induced by the pesticides in Tradescantia and those induced in Vicia faba indicates clearly that the cells of Vicia faba are much more sensitive to pesticides than are those of Tradescantia. This is shown by the greater toxicity of a given pesticide concentration to Vicia faba cells, and the higher frequency of chromosomal aberrations in Vicia faba following treatment.

Sparrow and Evans (1961) found a high correlation between nuclear volume, and nuclear DNA content with radiosensitivity in higher plants. According to their data, Tradescantia is more radiosensitive than Vicia faba. But it is important to remember that we are dealing with two entirely different systems, namely a chemical versus an ionizing one. Some of the criteria which make chemical mutagenesis different from radiation experiments are: (1) The uptake of the chemical, (2) The interaction of the chemical with the intracellular components, (3) The non-random nature of the effect of the chemical. Root tips of Tradescantia, unlike those of Vicia faba are protected by a thick root cap. This thick root cap affects the penetration of the pesticides into the cells.

Enzymes play a major role in maintaining the numerous biochemical processes of a cell. The application of pesticides may destroy, inhibit, or enhance the synthesis of certain enzymes and so may upset the cell system entirely. In this study the chemical mutagen ethylmethane sulfonate (EMS) has been used for comparative purposes. Recently, Scalera and Ward (1971) have shown that the primary reaction of EMS is the alkylation of guanine in the N-7 position. Ehrenberg et al. (1961) and Moutschen-Dahmen, J. and Moutschen-Dahmen, M. (1963) have reported that EMS produces few chromosomal aberrations in Hordeum vulgare and Vicia faba. In the present study, EMS produced translocations with an appreciable frequency in the treatment with the longest duration (12 hours), both in Tradescantia and Vicia faba. This discrepancy could be due to different procedures used in the experiments: In the studies referred to above, dry seeds were treated,

whereas in this study, roots of Tradescantia and Vicia faba were treated directly.

Chromosome breakage induced by a number of chemicals has been shown to be specific and localized. Maleic hydrazide (McLeish, 1952), 8-ethoxy caffeine (Kihlman, 1962), and Myleran (Moutschen-Dahmen, J., and Moutschen-Dahmen, M., 1958b) have been shown to induce breaks preferentially at different points in chromosomes of Vicia faba. Natarajan and Upadhya (1964), using Vicia faba as their test material showed that EMS (which attacks guanine) and hydroxylamine (which attacks cytosine) produced chromosome breaks which were highly specific and localized, coinciding with the known heterochromatic regions of the chromosomes of Vicia faba. Natarajan and Ahnström (1969) have shown that Nigella damascena chromosomes (completely devoid of heterochromatin) are relatively insensitive to all mutagenic agents except high LET (linear energy transfer) irradiation. The obvious differences between euchromatin and heterochromatin in connection with the production of aberrations are: (1) the condensed state of the heterochromatin during the major part of the interphase, (2) the association of heterochromatic regions either between homologous or non-homologous chromosomes to form chromocentres and, (3) late replication of DNA in heterochromatic regions.

In the present study, the most predominant types of chromosome aberrations observed were metaphase, anaphase and telophase fragments.

The frequency of chromosome breaks observed in Vicia faba was much higher than in Tradescantia. The conclusion which could be drawn from these results is that Vicia faba chromosomes, being rich in heterochromatic regions, are more sensitive to pesticides than those of Tradescantia.

The higher sensitivity of meiotic cells compared to somatic cells exposed to the same pesticide (for example, Phosdrin), in Vicia faba could be attributed to: (1) differential penetration of the pesticide into the root tip cells and pollen mother cells, (2) a long interphase period between the last premeiotic and first meiotic divisions in pollen mother cells and, (3) the persistence of the chemical on the inflorescence after spray treatment. In root tip treatment, however, the chemical is removed after a specific time period.

The results obtained in this investigation indicate clearly that lethal sensitivities and mutational susceptibilities tend to differ enormously, not only from species to species but even amongst the different tissues of an individual organism. Once the chemical penetrates the cell or attacks the genome, differences among species become evident. In one organism, the mutagen may rapidly be rendered nonmutagenic by metabolic processes which convert the compound to an inactive substance; in another species, the same compound may not be metabolized at all. Or, a nonmutagen may be converted to a mutagenic substance in one organism, whereas another species may metabolize the chemical by various pathways,

or not at all, so that the activation does not occur. Also, repair of genetic damage may differ between species.

The results of the present study clearly establish that the pesticides investigated are capable of inducing chromosomal aberrations both in Tradescantia and Vicia faba. Chromosomal aberrations are to be regarded as a sign of potential danger. Of the three pesticides used, Panogen 15--a methyl mercury compound--has been found to be the most deleterious to both the Tradescantia and Vicia faba genomes. For most pesticides, there is not much evidence, to the present time, of detrimental effects on man. But with mercury, the hazardous effects on man are already well documented. Can deleterious effects be reduced, or eliminated, without foregoing the benefits of pesticides? It is likely that they can be, in many cases, simply by selecting alternative pesticides which are substantially less hazardous to man and his environment.

SUMMARY

The overwhelming success of high crop production through the use of pesticides has overshadowed any side effects that pesticides may produce. However, the fact remains that a number of pesticides have deleterious effects on the genetic constitution of organisms.

1. The present study is an effort to elucidate the cytological effects of three pesticides on Tradescantia clone 02 and Vicia faba. The pesticides tested are Phosdrin (insecticide), Bladex (herbicide) and Panogen 15 (fungicide). Ethyl methane sulfonate (EMS) and colchicine (recognized chemical mutagens) have been used for comparative purposes. Three concentrations of Phosdrin and Bladex (200 p.p.m., 400 p.p.m., and 600 p.p.m.) and three treatment times (3 hours, 6 hours, and 12 hours) have been used to treat root tips of seedlings of Tradescantia and Vicia faba. Since Panogen 15 treatments were highly toxic to the cells the concentrations were reduced to 1 p.p.m., 2 p.p.m., and 5 p.p.m. and the duration of treatment to 1, 2 and 3 hours for this pesticide. Some 2500-3000 cells were observed for chromosome abnormalities for each pesticide. Spray treatments for meiotic studies were carried out for Vicia faba only. Plants were sprayed with 200, 400, and 600 p.p.m. of Phosdrin and Bladex.

Individual 2 X 2 contingency χ^2 tests were carried out to compare data from each treatment with the control data at 0.05, 0.01, and 0.001 levels of probability.

2. The pesticides tested did not induce any morphological mutations in the treated generation. Higher concentrations and longer durations of treatment with Panogen 15 were quite toxic to the whole root system of Tradescantia and Vicia faba.

Spraying with Phosdrin produced brown spots on the leaf margins of Vicia faba. Spraying with the same concentrations of Bladex was found to be lethal to the plants. The malformations observed could be attributed to physiological rather than genetic damage.

3. An increase in the concentration of Phosdrin increased the frequency of chromosomal aberrations in Tradescantia root tips for the 3 and 6 hour treatment periods. When the treatment time was increased to 12 hours the trend was reversed. The types of chromosomal aberrations observed were comparable to those produced by EMS.

4. An increase in the duration of treatment with Bladex seemed to decrease the percentage of aberrations produced in Tradescantia. Bladex, on the whole, produced a higher percentage (3.47%) of chromosome aberrations in root tip cells of Tradescantia than did Phosdrin (2.56%).

5. The different types of chromosomal aberrations produced by Phosdrin in Vicia faba roots were similar to those produced in Tradescantia. However, the frequency of anaphase fragments was

higher in Vicia faba. The data on the distribution and frequency of chromosomal aberrations induced by Phosdrin in Vicia faba root tips indicated that each treatment deviated significantly (probability < 0.001) from the control.

6. The percentage of chromosomal aberrations induced by Bladex in Vicia faba was lower than that caused by EMS and Phosdrin. This could be due to extreme toxicity of the herbicide Bladex to Vicia faba cells. The types of chromosome aberrations observed were: metaphase fragments, anaphase fragments, telophase fragments, multipolar anaphases, and lagging chromosomes.

7. The cytological abnormalities induced by Panogen 15 in root tips of Tradescantia are comparable to those produced by colchicine, namely: c-mitoses, multinucleate cells and polyploidy. The most characteristic features observed were differential staining of chromosomes and the presence of transverse interconnections within the chromatids of each chromosome.

8. Panogen 15 caused c-mitoses in Vicia faba root tips also. A 3-hour treatment period was more toxic to the root tips of Vicia faba than to those of Tradescantia. Transverse striations between the chromatids were also observed. The possible mode of action of this fungicide has been discussed.

9. Phosdrin caused chromosome abnormalities in the meiotic cells of Vicia faba. The most frequent types of chromosome irregularities were: extreme chromosome stickiness, fragments, bridges, and unequal

and asynchronous division of the chromosomes at anaphase II. At all the concentrations tested, the frequency of chromosomal aberrations produced by Phosdrin differed significantly from that of the control.

10. A comparison between the chromosome abnormalities induced in Vicia faba and Tradescantia indicated clearly that the cells of Vicia faba were much more sensitive to the pesticides than those of Tradescantia. Moreover, germ cells were more susceptible to pesticides than were the somatic cells. A possible explanation for the different sensitivities of these species has been discussed. The conclusion drawn is that lethal sensitivities and mutational susceptibilities tend to differ enormously, not only from species to species, but even between the different tissues of an individual organism.

From the foregoing, it can be concluded that Phosdrin and Bladex are effective chromosome breaking agents, whereas Panogen 15 is a potent c-mitotic agent. Of the three pesticides tested, Panogen 15, a methyl mercury fungicide, was found to be the most deleterious to the genomes of Tradescantia and Vicia faba.

BIBLIOGRAPHY

Amer, S. M. 1965. Cytological effects of pesticides. I. Mitotic effects of N-methyl-1-naphthyl carbamate "Sevin". Cytologia 30: 175-181.

Amer, S. M., and Ali, E. M. 1968. Cytological effects of pesticides. II. Meiotic effects of some phenols. Cytologia 33: 21-33.

Amer, S. M., and Farah, O. R. 1968. Cytological effects of pesticides. III. Meiotic effects of N-methyl-1-naphthyl carbamate "Sevin". Cytologia 33: 337-344.

Amer, S. M., and Ali, E. M. 1969. Cytological effects of pesticides. IV. Mitotic effects of some phenols. Cytologia 34: 533-540.

Ammini, C. 1968. Chromosome aberrations induced by maleic hydrazide in Rhoeo discolor Hance. Sci. and Cult. 34: 490-491.

Arnason, T. J., Mohammad, L., Koehler, D., and Rennerberg, F. M. 1962. Mutation frequencies in barley after treatment with gamma radiation, ethyleneimine, ethylmethane sulphonate and maleic hydrazide. Can. J. Genet. Cytol. 4: 172-178.

Auerbach, C., and Robson, J. M. 1944. Mutation frequency chemically induced. Production of mutations by allyl-isothiocyanate. Nature 154: 81.

Canvin, D. T., and Friesen, G. 1959. Cytological effects of CDAA and IPC on germinating barley and peas. Weeds 7: 153-156.

Carson, R. 1962. Silent Spring. Houghton-Mifflin, Boston. 368 pp.

Compton, W. 1952. The effect of maleic hydrazide on growth and cell division in Pisum sativum. Bull. Torrey Bot. Club 79: 205-211.

Croker, B. H. 1953. Effects of 2,4-dichlorophenoxy acetic acid and 2,4,5-trichlorophenoxy acetic acid on mitosis in Allium cepa. Bot. Gaz. 114: 274-283.

Curley, A., Sedlak, V. A., Girling, E. F., Hawk, R. E., and Barthel, W. F. 1971. Organic mercury identified as the cause of poisoning in humans and hogs. Science 172: 65-67.

Darlington, C. D., and McLeish, J. 1951. Action of maleic hydrazide on the cell. Nature 167: 407-408.

Datta, N. 1966. Cytological effects of gammexane on somatic chromosomes of Urginea coromandeliana Hook. F. Curr. Sci. 35: 75-76.

Derscheid, L. A., Stahler, L. M., and Kratochvil, D. E. 1952. Differential responses of barley varieties to 2,4-dichlorophenoxyacetic acid (2,4-D). Agron. J. 44: 182-187.

Doxey, D. 1949. The effect of isopropylphenyl carbamate on mitosis in rye and onion cells. Ann. Bot. 13: 329-335.

Ehrenberg, L., Gustafsson, A., and Lundquist, V. 1961. Viable mutants induced in barley by ionizing radiations and chemical mutagens. Hereditas 47: 243-283.

Ennis, W. B., Jr. 1948. Some cytological effects of O-isopropyl N-phenyl-carbamate upon Avena. Amer. J. Bot. 35: 15-21.

Epstein, S., Andrea, J., Jaffe, H., Joshi, S., Falk, H., and Mantel, N. 1967. Carcinogenicity of the herbicide maleic hydrazide. Nature 215: 1388-1390.

Fahmy, F. Y. 1951. Cytogenetic analysis of the action of some fungicide mercurials. Ph.D. Thesis, Inst. Genet., Univ. of Lund, Sweden.

Fahmy, O. G., and Fahmy, M. J. 1957. Comparison of chemically and X-ray induced mutations in Drosophila melanogaster. Nature 117: 986-997.

Ficsor, G., Janca, F. C., and Hook IV, C. 1970. Tests for mutagenicity using nitrosoguanidine, DDT, and Captan in maize. Environ. Mutagen Soc. 3: 22.

Fiskesjö, G. 1969. Some results from Allium tests with organic mercury halogenides. Hereditas 62: 314-322.

Fiskesjö, G. 1970. The effect of two organic mercury compounds on human leucocytes in vitro. Hereditas 64: 142-146.

Friesen, H. A., Baenziger, H., and Keys, C. H. 1964. Morphological and cytological effects of Dicamba on wheat and barley. Can. J. Plant Sci. 44: 288-294.

Froese-Gertzen, E. E., Konzak, C. F., Nilan, R. A., and Heiner, R. E. 1964. The effect of ethylmethane sulfonate on the growth response, chromosome structure and mutation rate in barley. Radiat. Bot. 4: 61-69.

Gentner, W. A., and Burk, L. G. 1968. Gross morphological and cytological effects of Nitralin on corn roots. Weed Sci. 16: 259-260.

George, M. K., Aulakh, K. S., and Deshi, S. 1970. Morphological and cytological changes induced in barley (Hordeum vulgare) seedlings following seed treatment with fungicides. Can. J. Genet. Cytol. 12: 415-419.

Gibson, P. B., and Beinhart, G. 1969. Abnormal meiosis in clover plants treated with organic phosphate pesticides. Bull. South Carolina Acad. Sci. 31: 38.

Gifford, E. M. 1956. Some anatomical and cytological responses of barley to maleic hydrazide. Amer. J. Bot. 43: 72-80.

Golomb, H. M., and Bahr, G. F. 1971. Scanning electron microscopic observations of surface structure of isolated human chromosomes. Science 171: 1024-1026.

Graf, G. E. 1957. Chromosome breakage induced by X-rays, maleic hydrazide and its derivatives. J. Hered. 48: 155-159.

Grant, W. F., and Harney, P. M. 1960. Cytogenetic effects of maleic hydrazide treatment of tomato seed. Can. J. Genet. Cytol. 2: 162-174.

Greulach, V. A., and Atchison, E. 1950. Inhibition of growth and cell division in onion roots by maleic hydrazide. Bull. Torrey Bot. Club 77: 262-267.

Grinikh, L. I. 1969. Changes in effectiveness of ethylmethane sulfonate on Crepis capillaris seeds. Genetika 5: 19-31.

Haddow, A., and Ross, W. C. J. 1956. Tumour growth-inhibitory alkyl sulphonates. Nature 177: 995-996.

Kihlman, B. A. 1962. The production of chromatid aberrations by 5-fluorodeoxyuridine alone and in combination with X-rays and 8-ethoxycaffeine. Caryologia 15: 261-277.

Kostoff, D. 1931. Heteroploidy in Nicotiana tabacum and Solanum melongena caused by fumigation with nicotine sulphate. Bull. Soc. Bot. Bulgarie 4: 87-92.

Kostoff, D. 1939. Effect of the fungicide "Granosan" on atypical growth and chromosome doubling in plants. Nature 144: 334.

Kostoff, D. 1948. The atypical growth, abnormal mitosis, polyploidy and chromosome fragmentation induced by hexachlorocyclohexane. Nature 162: 845-846.

Levan, A. 1938. The effect of colchicine on root mitoses in Allium. Hereditas 24: 471-486.

Levan, A. 1954. Colchicine induced c-mitosis in two mouse ascites tumors. Hereditas 40: 1-64.

Liang, G. H. L., Feltner, K. C., Liang, Y. I. S., and Morrill, J. L. 1967. Cytogenetic effects and responses of agronomic characters in grain sorghum (Sorghum vulgare Pers.) following Atrazine application. Crop Sci. 7: 245-248.

Loveless, A., and Howarth, S. 1959. Mutation in bacteria at high levels of survival by ethylmethane sulphonate. Nature 184: 1780-1782.

MacFarlane, E. W. E. 1950. Somatic mutations produced by organic mercurials in flowering plants. Genetics 35: 122-123.

MacLeod, R. D. 1969. Some effects of 2,4,5-trichlorophenoxy acetic acid on the mitotic cycle of lateral root apical meristems of Vicia faba. Chromosoma 27: 327-337.

Mazia, D. 1955. The organization of the mitotic apparatus. Symp. Soc. Exp. Biol. 9: 335-357.

Mazia, D. 1961. Mitosis and the physiology of cell division, 3: 77-412. In Brachet, J., and Mirsky, A. E. (eds.), The Cell, Academic Press, New York.

McLeish, J. 1952. The action of maleic hydrazide in Vicia. Heredity 6 (Suppl.): 125-147.

Morgan, T. H. 1914. The failure of ether to produce mutations in Drosophila. Amer. Natur. 48: 705-711.

Morrison, J. W. 1962. Cytological effects of the herbicide "Avadex". Can. J. Plant Sci. 42: 78-81.

Moutschen-Dahmen, J., et Moutschen-Dahmen, M. 1958b. L'action de myleran sur les chromosomes chez Hordeum sativum et chez Vicia faba. Hereditas 44: 415-446.

Moutschen-Dahmen, J., et Moutschen-Dahmen, M. 1963. Intéractions ioniques dans les effets radiomimétiques du méthane sulfonate d'éthyle (EMS) sur les chromosomes de Vicia faba. Radiat. Bot. 3: 297-310.

MUhling, G. N., Van't Hof, J., Wilson, G. B., and Gingsby, B. H. 1960. Cytological effects of herbicidal substituted phenols. Weeds 8: 173-181.

Muller, H. J. 1927. Artificial transmutation of the gene. Science 66: 84-87.

Natarajan, A. T., and Upadhya, M. D. 1964. Localized chromosome breakage induced by ethyl-methane-sulfonate and hydroxylamine in Vicia faba. Chromosoma 15: 156-169.

Natarajan, A. T., and Ahnström, G. 1969. Heterochromatin and chromosome aberrations. Chromosoma 28: 48-61.

Prasad, I., and Pramer, D. 1968. Genetic effects of Ferbam on Aspergillus niger and Allium cepa. Phytopathology 58: 1188-1189.

Prasad, I., and Pramer, D. 1969. Cytogenetic effects of Propanil and its degradation products on Allium cepa L. Cytologia 34: 351-352.

Pridham, A. M. S. 1947. Effect of 2,4-D on bean progeny seedlings. Science 105: 412.

Ramel, C. 1969. Genetic effects of organic mercury compounds. I. Cytological investigations on Allium roots. Hereditas 61: 208-230.

Ramel, C., and Magnusson, J. 1969. Genetic effects of organic mercury compounds. II. Chromosome segregation in Drosophila melanogaster. Hereditas 61: 231-254.

Rao, R. N., and Natarajan, A. T. 1965. Mutagenicity of alkyl alkanesulfonates in barley. Mutat. Res. 2: 132-148.

Reddy, M. V., and Rao, R. B. V. 1969. The cytological effects of insecticides (Dimecron-100 and Rogar-40) on Vicia faba L. Cytologia 34: 408-417.

Rossman, E. C., and Sprague, G. F. 1949. Effect of 2,4-D on yields of maize in the succeeding generation after treatment. Plant Physiol. 24: 770-773.

Sass, J. 1937. Histological and cytological studies of ethyl mercury phosphates. Phytopathology 27: 95-99.

Satpathy, D., and Arnason, T. 1969. Pretreatment temperature effects on mutation frequency in ethylmethane sulfonate treated barley. Can. J. Genet. Cytol. 11: 522-530.

Sawamura, S. 1964. Cytological studies on the effect of herbicides on plant cells in vivo. I. Hormonic herbicides. Cytologia 29: 86-102.

Sawamura, S. 1965. Cytological studies on the effect of herbicides on plant cells in vivo. II. Non-hormonic herbicides. Cytologia 30: 325-348.

Sax, K., and Sax, H. J. 1968. Possible mutagenic hazards of some food additives, beverages and insecticides. Jap. J. Genet. 43: 89-94.

Scalera, S. E., and Ward, O. G. 1971. A quantitative study of ethyl methanesulfonate-induced alkylation of Vicia faba DNA. Mutat. Res. 12: 71-79.

Scholes, M. E. 1955a. The effects of chlordane and toxaphene on mitosis in roots of the onion (Allium cepa). J. Hort. Sci. 30: 12-24.

Scholes, M. E. 1955b. The effects of aldrin, dieldrin, isodrin, endrin and DDT on mitosis in roots of the onion (Allium cepa). J. Hort. Sci. 30: 181-187.

Smith, L. H., and Harrison, C. M. 1962. Effect of 2,4-dichlorophenoxyacetic acid on seedling development and uptake and distribution of calcium and phosphorus in barley. Crop Sci. 2: 31-34.

Sparrow, A. H., and Evans, H. J. 1961. Nuclear factors affecting radiosensitivity. I. The influence of nuclear size and structure, chromosome complement, and DNA content. Brookhaven Symp. Biol. 14: 76-100.

Storey, W. B., Jordan, L. S., Mann, J. D. 1968. Carbamate herbicides--new tools for cytological studies. Calif. Agr.: 12-13.

Stroyev, V. S. 1968. Cytogenetic activity of the herbicides--simazine and maleic acid hydrazide. Genetika 4: 130-134.

Swaminathan, M. S., Chopra, V. L., and Bhaskaran, S. 1962. Chromosome aberrations and the frequency and spectrum of mutations induced by ethylmethane sulfonate in barley and wheat. Indian J. Genet. Plant Breed. 22: 192-207.

Unrau, J. 1953. Cytogenetic effects of 2,4-D on cereals. Canadian Seed Growers' Assoc. Ann. Rept., pp. 25-28.

Unrau, J. 1954. Cytogenetic effects of 2,4-D on cereals. Canadian Seed Growers' Assoc. Ann. Rept., pp. 37-39.

Vaarama, A. 1947. Experimental studies on the influence of DDT insecticide upon plant mitosis. Hereditas 33: 191-219.

Vaurughese, G., and Swaminathan, M. S. 1970. A comparison of the frequency and spectrum of mutations induced by gamma rays and ethylmethane sulfonate in wheat. Indian J. Genet. Plant Breed. 28: 158-165.

Veleminsky, J., and Gichner, T. 1963. Cytological and genetic effects of the insecticide Systox on Vicia faba L., and Arabidopsis thaliana L. (Heynh). Biol. Plant. 5: 41-52.

Wakonig, R., and Arnason, T. J. 1959. Chromosome breakage induced in Vicia faba root tips by 2,4,6-tri(ethyleneimino)-1,3,5-triazine. Can. J. Bot. 37: 403-411.

Wuu, K. D., and Grant, W. F. 1966a. Morphological and somatic chromosomal aberrations induced by pesticides in barley (Hordeum vulgare). Can. J. Genet. Cytol. 8: 481-501.

Wuu, K. D., and Grant, W. F. 1966b. Induced abnormal meiotic behavior in a barley plant (Hordeum vulgare) with the herbicide Lorox. Phyton 23: 63-67.

Wuu, K. D., and Grant, W. F. 1967a. Chromosomal aberrations induced in somatic cells of Vicia faba by pesticides. Nucleus 10: 37-46.

Wuu, K. D., and Grant, W. F. 1967b. Chromosomal aberrations induced by pesticides in meiotic cells of barley. Cytologia 32: 31-41.

Yaker, N. 1952. Mitotic disturbances caused by chloranil. Amer. J. Bot. 39: 540-546.

Zeisel, S. 1883. Über Colchicn und Colchicein. Monatsh Chem. 4: 162-164.