Morphological and Cytogenetic Effects of Isotox 25 Seed Treater (F) (Lindane and Captan) on Roots and Chromosomes of Rye (*Secale cereale* L.)

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The use of pesticides has increased enormously over the last 35 years. World-wide, we now use about 2 billion kilograms per year of 40,000 different pesticides (Miller 1982). Many of these chemical compounds exhibit detrimental effects on organisms other than the pests they were designed to control. For example, 2,4-D, an herbicide used for weed control, causes chromosome aberrations and abnormal mitosis in cotton (Basler and Nakazawa 1961), onion (Ryland 1948) and horsebeans (Sawamura 1963). Ferbam, a fungicide used on fruits, nuts and tobacco, causes chromosomal damage on onion root tips (Prasad and Pramer 1968). Vitavax-200 and Dithane S-60 are also well known pesticides used to control pathogenic fungi affecting wheat. Both reduce the mitotic index and greatly increase the frequency of chromosomal bridge formation during anaphase in bread wheat (Nahla and Soliman 1980). Njagi and Gopalan (1981) showed that the insecticides summethion, lannate, Carbicron, Thiodan and Kelthane, the fungicides Diathane M-45 and Aldrin, and the herbicides Ramrod, Lasso, Round-up and Gammoxone all produce cytogenetic changes in *Vicia faba*. Hocking and Thomas (1979) reported that the fungicide Captan inhibited root formation in cuttings of ornamental plants. Lindane was shown to be phytotoxic to 3-day old garden pea seedlings (Charnetski *et al.* 1973).

The present study reports on the effects of a proprietary seed treatment product containing both an insecticide (Lindane) and a fungicide (Captan) on rye roots.

Materials and methods

Clean dry seeds of rye (*Secale cereale* L.) var. Snoopy were used throughout this study. The seeds were dusted at recommended rates for cereals with Ortho's Isotox 25 Seed Treater (F). The active ingredients include 25% Lindane (gamma isomer of benzene hexachloride, CAS No. 58-89-9) and 12.5% Captan (N-(trichloromethyl)thio-4-cyclohexene-1,2-dicarboximide, CAS No. 133-06-2). The treated and control seeds (100 each) were germinated at 20°C in darkness.

Ten root tips from treated and control were removed when 1-3 cm long and placed directly in ice water at 5°C for 24 h to accumulate somatic cells in metaphase (Nakata *et al.* 1977). The root tips were treated to saturated bromonaphthalene for 1 h at 20°C, stained in 3% aceto-orcein for 23 h at 5°C and rinsed several times in running tap water. Chromosome spreads were made using the squash technique

on subbed slides and an Ikonen micro-press (Winnipeg Man.) was used to further flatten and spread the chromosomes. They were made permanent with Histo-Freeze (Fisher Cat. No. 12-645-30), air dried, soaked in xylene and mounted with Pro-Texx.

Results and discussion

The rye seeds treated with Isotox-25 showed a slightly higher germination rate (91.3%) than the controls (86.3%). Six days after first emergence of the radicle, the average length of the untreated roots (92.3 mm) was much greater than the treated roots (7.7 mm). The ends of the treated roots were swollen and club-shaped just proximal to the root tip and measured about 1.6 mm in diameter. The diameter of the untreated root tips was near 0.3 mm. The clubbing did not appear until 2 days after emergence of the radicle.

The diploid number of chromosomes for rye is 14. Minor variations from this (for example, 12 or 13) in the ten control slides represent chromosome loss due to the cytological technique and not the normal condition in the living plant. The ten slides of treated root tips show cells exhibiting large and varying numbers of chromosomes ranging from 14 to 190. For example, slide No. 6 showed cells with 28, 85, 49, 96 and 67 chromosomes. Table 1 indicates chromosome numbers of 5 cells from one control slide (0) and ten treated slides (1-10). The modal ploidy is probably more useful than the mean ploidy level (Eggen 1970). Many of the cells contained chromosomes near the octoploid number of $8n=56$.

Since the chromosome number of a cell doubles with each division without cytokinesis, one would expect multiples of 14 (28, 56, 112, 224) in the treated cells. Such is not the case as most of the cells contained numbers of chromosomes other than exact multiples of the diploid condition. Perhaps some cytokinesis occurs that prevents their recovery but it was not observed in this material due to pretreatment with cold and bromonaphthalene.

It is suggested that Lindane is the active ingredient responsible for the above observations. This is supported by the work of others (Epstein and Legator 1971,

Table 1. Chromosome counts of five cells from one control (0) and ten treated (1-10) root tips

Slide no.	Chromosome numbers of five cells	Modal ploidy	Mean	Mean ploidy
0	14, 14, 14, 12, 14	2n	14	2n
1	55, 37, 28, 53, 76	8n	50	8n
2	38, 51, 49, 55, 32	8n	45	7n
3	53, 29, 54, 61, 50	8n	49	7n
4	74, 27, 51, 55, 83	8n	58	9n
5	38, 54, 61, 14, 40	8n	41	6n
6	28, 85, 49, 96, 67	14n	65	10n
7	53, 50, 28, 51, 51	8n	46	7n
8	48, 54, 71, 163, 53	8n	78	12n
9	62, 52, 31, 87, 49	9n	56	8n
10	27, 28, 56, 190, 37	4n	68	10n

Vintila *et al.* 1979) who have shown that Lindane causes many types of chromosomal aberrations in a wide variety of plant and animal species. The thickening or "clubbing" of the roots treated with Lindane was noted in peas by Charnetski *et al.* (1973), in wheat and broad bean by Lazar-Keul *et al.* (1979), in corn, wheat, sunflowers and cabbage by Kostoff (1949) and in onion and strawberries by Scholes (1953). Apparently Lindane acts to prevent normal microtubular formation (Jeanne 1978) and cell wall development (Charnetski *et al.* 1973). This leads to abnormal mitosis (resembling C-mitosis) which produces large heteroploid cells and results in short swollen root tips. Recovery from such treatment was not investigated in the present work.

Lindane combined with Captan and used as a seed treater to control insects and fungi on the seeds of many plants has been shown, in the present work on rye roots, to decrease cell elongation, increase cell size and root diameter and produce high levels of polyploidy.

Summary

Isotox 25 Seed Treater (F) was applied at recommended rates to dry seeds of rye which were allowed to germinate. Roots from treated seeds were short and thickened near the apex. Cells of root tips showed greatly enlarged cells possessing nuclei exhibiting various levels of polyploidy.

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