CARBAMATE HERBICIDES—
new tools for cytological studies

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CYTOLOGY (the branch of biology dealing with studies of cell structure and function under the microscope) is important to both plant and animal scientists, because cytological studies disclose useful information on cell organization and behavior under both normal and artificially induced conditions. Plant physiologists in the Department of Horticultural Science, Riverside, are actively engaged in research on herbicides, with special emphasis on selective elimination of weeds from plantings of crop plants. In some experiments, the effects of herbicides become immediately apparent in either the weeds or the crop plants, or in both. In other experiments, damage to both weeds and crop plants remains concealed for a long time before it becomes evident. Such variable and often unpredictable behavior explains the interest of weed control physiologists in the mode of action of selective herbicides upon the species under study. One of the more promising approaches to mode-of-action studies is the cytological examination of herbicidal effects upon individual cells, especially dividing cells in root tissues.

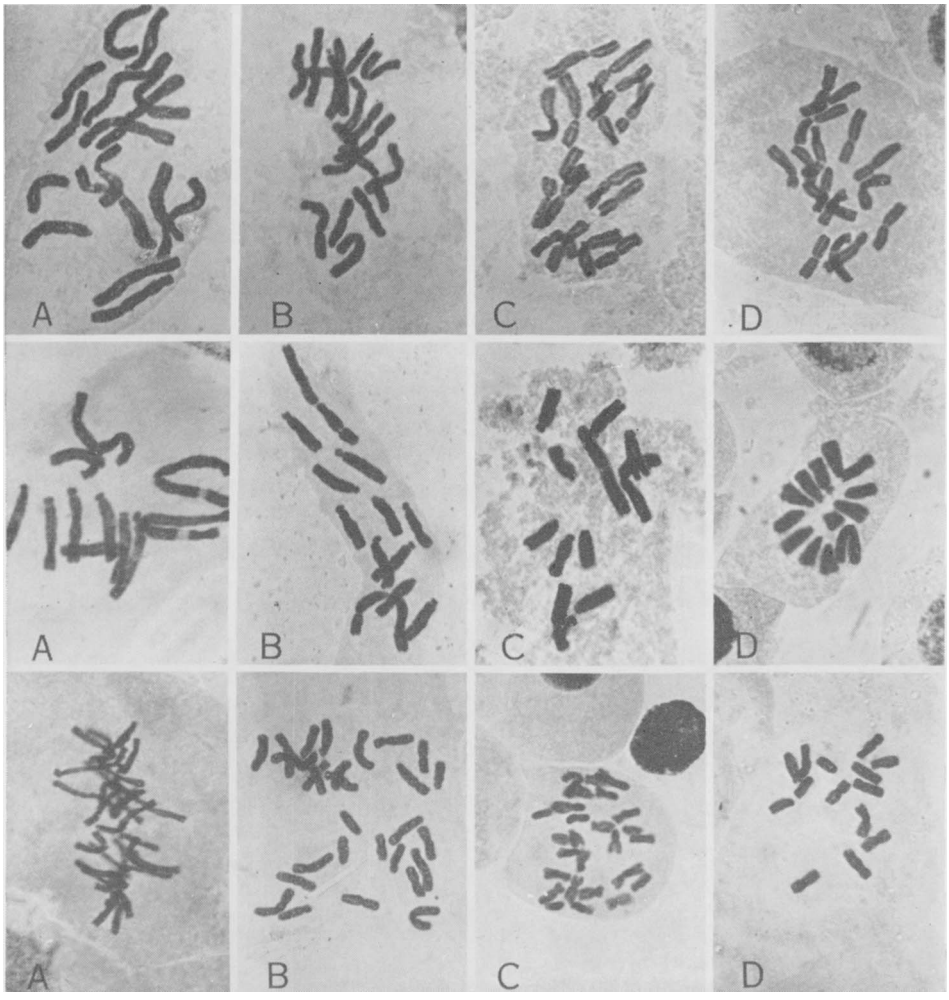
Geneticists and plant breeders obtain cytological information largely from chromosomes, the bearers of heredity. Much can be learned from studies of numbers, sizes, structure, and behavior in cell divisions, both in vegetative tissues and in tissues which develop into germ cells. Root tips are the most convenient material for studying vegetative cell chromosomes in most plant species.

Vegetative cell chromosomes of many species of plants are long and stringy at the best stage for study—often with parts touching and intercrossing, and tending to resist spreading on the microscope slide, even when the root tips are squashed. This resistance to spreading makes counting the chromosomes and studying their sizes and structural features difficult and time-consuming, and sometimes quite impossible.

A number of chemicals presently in use are effective in condensing chromosomes, thereby making their study easier. The most widely used are colchicine and 8-hydroxyquinoline. Less widely used are paradichlorobenzene, acenaphthene, chloral hydrate, and maltose. These chemicals produce desirable results in many, but not all, species of plants.

Cytological studies on the mode of action of one family of chemicals, the carbamates, disclosed that several members are highly effective in contracting chromosomes, and, to some extent, in improving their "spreadability" on the microscope slide. Members that have been tested and found to produce desirable results are: *O*-isopropyl N-phenylcarbamate (IPC); isopropyl N-(3-chlorophenyl)-carbamate (CIPC); 2,3-dichlorobenzyl methyl carbamate (2, 3 DMC); 3,4-dichlorobenzyl methyl carbamate (3,4 DMC); methyl-4-methoxycarbonylaminobenzesulphonylcarbamate (MB 9555); and benzyl adenine (BA).

These chemicals have been tested on more than 20 species of plants in such diverse groups as gymnosperms, monocotyledons, and dictotyledons—always with favorable results. The principal effects are rapid inhibition of cell activity, and contraction of the chromosomes, prob-



Mitotic metaphase chromosomes in excised root tips of onion, top row; broad bean, center row; and spider plant, bottom row. Chromosomes were condensed by treating root tips in various dilute solutions of carbamate compounds for various periods of time, and are shown in comparison with chromosomes in untreated root tips (all photos at same magnification).

UPPER ROW: Onion (2n = 16)—A. No treatment; B. 5 ppm IPC, 2 hr; C. 5 ppm CIPC, 2 hr; and D. 5 ppm BA, 2 hr.

CENTER ROW: Broad bean (2n = 12)—A. No treatment; B. 5 ppm 3,4 DMC, 2 hr; C. 5 ppm 2,3 DMC, 2 hr; and D. 40 ppm IPC, 1 hr.

LOWER ROW: Spider plant (2n = 28)—A. No treatment; B. 5 ppm MB9555, 3 hr; C. 5 ppm BA, 3 hr; and D. Haploid cell (n = 14), 10 ppm IPC, 2 hr.

ably in all stages of cell division. The degree of contraction depends a good deal on the concentration of the solution used and duration of treatment prior to killing the root tips for staining. The effects produced by some of these chemicals on root tip chromosomes of three common plants at metaphase (the stage of division at which they are most condensed under natural conditions) are shown in the photo. The plants are: onion (*Allium cepa*, 2n = 16), broad bean (*Vicia faba*, 2n = 12), and spider plant (*Chlorophytum elatum*, 2n = 28).

The carbamates are white, crystalline solids of low solubility in water. Saturated solutions vary widely in concentration; they must be diluted for chromosome studies. Satisfactory dilutions lie between 5 and 40 ppm. Time of treatment is important, as the effective period for satisfactory results lies between 1 and 3 hours. Destruction of chromosomes occurs rapidly at concentrations exceeding 50 ppm, and ultimately at low concentrations if treatment time exceeds 4 to 5 hours. A suggested starting point from which one may vary concentration and treatment time either way is 10 ppm for 1 hour for excised root tips of most species of plants.

Two important precautions must be taken in using carbamates to pre-treat material for cytological studies. First, bottles, vials, and other glassware to be used must be scrupulously clean. Second, the chemical should be mixed directly with distilled water to make a saturated stock solution. Not even a trace of alcohol or other organic solvent should be used to dissolve the chemical before adding it to the water because the chromosomes may be destroyed rapidly even at high dilutions. The reason for this destruction is not known, but may involve either extremely rapid penetration of the chemical into the cell, or synergistic activity of the chemical and solvent. A stock solution in a well stoppered bottle was found to have lost none of its potency after two years at room temperatures in the laboratory.

Two advantages in the use of carbamates such as IPC and CIPC are their relative safety and economy. They have failed consistently to produce tumors of any sort on laboratory animals in exhaustive clinical experiments, and are regarded as noncarcinogenic. The purified chemicals cost about $3.00 per pound. The solubility of purified IPC is roughly 250 ppm. One gram, costing less than one cent, is sufficient to make up about 400 liters of saturated stock solution, or 10,000 liters of 10 ppm solution.

The carbamates investigated provide cytologists with a new tool for chromosome studies. Additional carbamates, as well as other presently unexplored groups of selective herbicidal chemicals, may produce comparable or different effects and become equally useful.

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Magnesium deficiency in cut-flower chrysanthemums

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A severe leaf yellowing, or chlorosis problem, has occurred in chrysanthemums at several commercial greenhouses in the San Francisco Bay Area. The disorder was found to be magnesium deficiency caused by high annual applications of potassium. Magnesium fertilization, even at high rates, was not effective in correcting the problem. Elimination of potassium fertilization gave immediate control.

THE CHLOROSIS discussed in this study appeared on rooted cuttings of chrysanthemums shortly after they were planted in ground beds. On young plants the symptoms were confined to the older leaves. These exhibited an interveinal yellowing, accompanied in advanced stages by a reddish-purple pigmentation of the leaf margins. Near bloom time the chlorosis progressed rapidly up the stem in severe cases, affecting most of the foliage and reducing the marketability of the cut flowers. Many varieties of standard chrysanthemums have been affected, including white and yellow Albatross, Indianapolis, Lavender Queen, Donolope, White Spider, Copperhead, Bunbu, and Iceberg.

The magnesium and potassium contents of leaves representing different stages of symptom development on White Albatross are shown in table 1. Chlorotic leaves contained less than 0.1 per cent magnesium. The percentage of magnesium found in the leaves with mild symptoms (0.06 per cent) is in good agreement with the magnesium critical level established by research on chrysanthemums at University of California, Los Angeles. The leaf analyses, leaf symptoms, and the progessive pattern of symptoms, from lower to upper leaves, all indicated a magnesium-deficiency problem.

The grower's fertilization program included annual applications of potassium at a very high rate. Continuous application of potassium to soils in amounts greatly exceeding crop requirements can lead in time to an imbalance of potassum and magnesium in the soil. Under such conditions, magnesium uptake by plants is restricted and magnesium deficiency can develop, even if the soil contains a normal amount of available magnesium.

Normal chrysanthemum leaf to left, as compared with magnesium deficient leaf to right.

In the case of these chrysanthemums, potassium had been applied since 1948 preplant to each crop—every 15 weeks at the rate of 1 to 1½ lbs of potassium sulfate per 100 sq ft. At this rate the annual application of potassium sulfate amounted to 3½ to 5¼ lbs per 100 sq ft. In addition, appreciable amounts of potassium were added in the form of barley