

stained with lead and/or uranyl acetate. The observations were carried out with Siemens Elmiskop IA electron microscope.

Results and discussion. Chromatic granules in *T. vaginalis* were present predominantly along and around the mastigont system (Figure 1). Their fine structure differed greatly depending on the method of fixation.

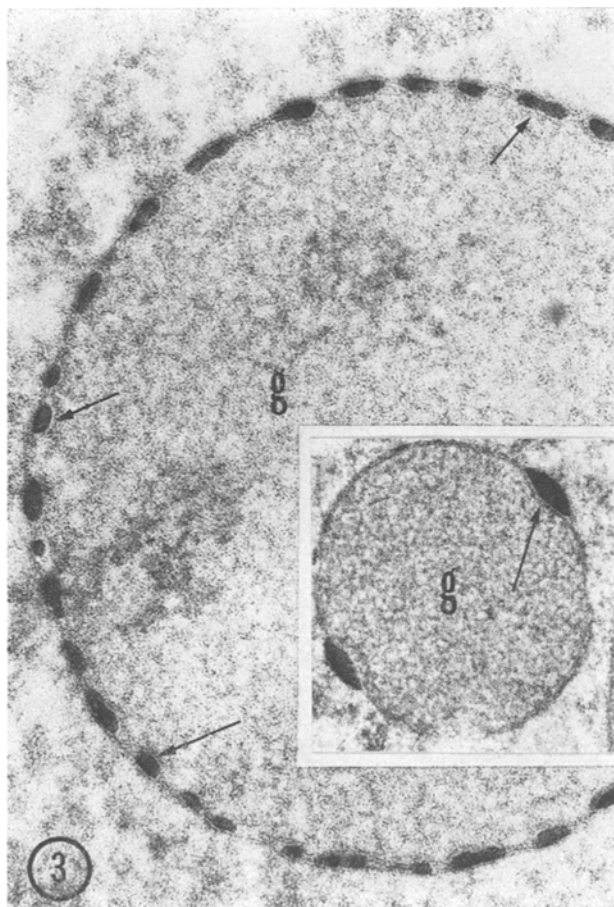


Fig. 3. Higher magnification of a chromatic granule (g) of *T. vaginalis* fixed with method 2. Numerous electron-opaque inclusions are visible in the envelope of the granule. The inclusions surrounded by an optically empty layer 30 Å thick are indicated by arrows. $\times 98,000$. Insert: Higher magnification of a chromatic granule (g) containing only 2 lens-shaped inclusions (arrow) in its envelope. Fixation with method 2. $\times 96,000$.

Chromatic granules fixed with method 1 were limited by a single trilaminar membrane which occasionally had only additional dark and light bands (Figure 2). After application of method 2, lens-shaped inclusions were found along the envelope of chromatic granules (Figures 1 and 3). Between inclusions, the envelope presented the usual aspect of a single trilaminar membrane. Each inclusion was covered on both sides with a 25–30 Å thick optically empty band belonging to the envelope of a granule (Figure 3). Inclusions were either homogenous or contained optically empty core; they were situated regularly but their length varied from one granule to another (compare Figure 3). Their width was more constant and ranged from 60–80 Å. Inclusions were seen in all cells fixed with method 2.

Fixation with 4% unbuffered OsO_4 permitted us to observe the characteristic inclusions in the envelope of chromatic granules, the as yet unknown structural detail. It is possible that higher percentage and lower pH value of osmium tetroxide used in method 2 prevented the extraction of some component responsible for the morphological appearance of inclusions. The reproducibility of findings suggests that inclusions may correspond to structures present *in vivo* in *T. vaginalis*. The finding of the inclusions does not facilitate the classification of chromatic granules or the understanding of their functional significance. Some ultramorphological similarities between chromatic granules and microbodies⁶ may be indicated. Inclusions described in the present paper may correspond to marginal plate or other component of microbodies. So the possibility arises that chromatic granules might be an equivalent of microbodies which were up to now identified in Protozoa on biochemical basis only⁶. To test this assumption, specific cytochemical reactions must be carried out.

Riassunto. Nel presente lavoro viene descritta l'ultrastruttura dei granuli cromatici di *Trichomonas vaginalis* Donné. A seguito di modificazioni alle metodiche della fissazione delle cellule per la microscopia elettronica sono state osservate delle inclusioni nella membrana di questi granuli non descritte in letteratura.

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Induction of Female Flowers on Male Plants of *Cannabis Sativa* L. by 2-Chloroethanephos-phonic Acid

Sex expression in *Cannabis sativa*, a dioecious annual, can be modified by temperature, day length, as well as exogenous application of auxin¹. The present investigation was undertaken to test whether Ethrel (2-chloroethanephosphonic acid), a recently recommended source of ethylene, could induce femaleness in male plants of *Cannabis sativa*.

Material and method. Seedlings of *C. sativa* were raised in earthen pots. They flowered 8 weeks after germination and their sexes were determined; 60 male plants were selected for treatment and the height and number of

vegetative and flowering nodes were recorded for each plant.

Three concentrations (240, 480 and 960 ppm) of Ethrel were applied in one foliar spray (using a hand sprayer) till the point of run-off; triton X-114 at 0.01% was used as wetting agent. 45 male plants received Ethrel; 15 received only triton X-114 (controls). These plants were kept under natural conditions obtaining during January–March, in the departmental botanical garden. The height and flower number were recorded every week following the treatment.

Results and discussion. A close-up of the terminal part of control plant is shown in Figure A. Ethrel-treated plants showed characteristic drooping and epinasty of leaves after the second day of spray. The higher the concentration, the more severe was the effect. However, 10 days after treatment these symptoms disappeared. Treated plants were shorter than controls and appeared bushy. This was due to reduction in internodal elongation, and not due to reduced node number. The new leaves were smaller than those on controls.

Plants treated with Ethrel showed female, intersexual and abnormal male flowers in the newly-formed nodes (Table). At 240 ppm only a few nodes bearing intersexual flowers and flowers with reduced number of stamens were noticed. In the latter only 1–3 stamens were observed as against the usual 5 in controls. No female flowers were formed (Table). Plants which received Ethrel at 480 ppm showed a larger number of nodes with female and intersexual flowers, and flowers with reduced number of stamens. All these types of flower occurred in the same cluster. A drastic decrease in the total number of flowers, and a high degree of feminization (Figure B) occurred at 960 ppm.

The female flowers which were induced on male plants were similar to control female flowers and set seeded fruits after hand pollination. The intersexual flowers represented stages of transformation of normal male flowers to female flowers. In some flowers the apex of anther had

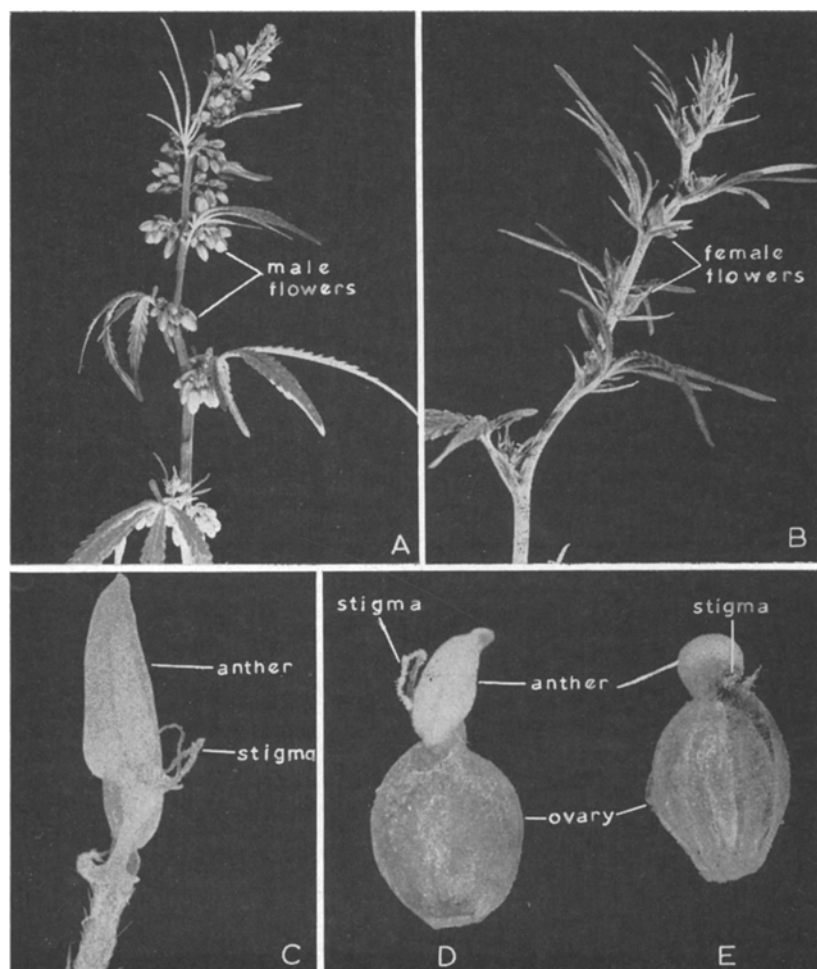
become stigma-like, and in others the base of the anther was transformed into an ovary and the terminal part showed rudimentary anther and stigma (Figures c, d and

Effect of Ethrel on flower sex-expression in male plants of *Cannabis sativa*

Treatment	Total no. of flowers produced in 5 nodes/plant ^a	% of ♂ flowers	% of ♀ flowers	% of flowers showing inter-sexed nature or reduced no. of stamens
Control	129.4	100	0	0
Ethrel 240 ppm	120.4	98.1	0	1.9
Ethrel 480 ppm	105.0	27.0	4.5	68.5
Ethrel 960 ppm	31.2	6.3	69.9	23.8

^a Average of 5 plants.

¹ J. HESLOP-HARRISON, Biol. Rev. Cambridge Phil. Soc. 32, 38 (1957).



(A) Terminal part of control male plant showing male flowers. $\times 0.3$.

(B) Male plant treated with 960 ppm of Ethrel has developed female flowers. $\times 0.3$.

(C, D and E) Flowers from Ethrel-treated male plants showing stages of transformation into female flowers. C, $\times 16.6$; D, $\times 9.3$; E, $\times 15$.

Ε). Intersexual flowers and flowers with reduced number of stamens bore pollen which were normal in shape and size. After the 4th week of treatment, plants started bearing normal male flowers. However, plants sprayed with 960 ppm of Ethrel took 6 weeks to revert to the production of normal flowers.

The data presented here demonstrate that the flower sex-expression of male plants of *Cannabis sativa* can be altered by treatment with Ethrel. It has been suggested that Ethrel decomposes in the plant tissues to release ethylene^{2,3}. It is recently claimed that some effects of auxin are exerted through an ethylene evolution mechanism⁴. It is likely that Ethrel (a source of ethylene) exerts its effect on sex-expression by manipulating the endogenous level of auxin⁵.

Zusammenfassung. Ethrel induziert auf männlichen Hanfpflanzen (*Cannabis sativa* L.) weibliche Blüten, welche nach der Bestäubung Früchte ausbilden.

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⁵ Acknowledgments: We are grateful to Prof. B. M. JOHRI for interest and facilities and to Amchem Products Inc., Ambler (Pa., USA), for a gift sample of Ethrel.

Catalase and Peroxidase Activity in Sugarcane Infected with Sugarcane Mosaic Virus

Virus infection usually brings about drastic changes in the physiological processes of the host cell. Among the physiological effects of virus infection on host plants, changes have been observed mostly in over-all respiratory activity¹. Changes in the activity of oxidative enzymes have also been studied both in fungus diseased² and virus infected plants^{3,4}. Very little work, however, has been done with mosaic infected sugarcane plants. YAMAFUGI et al.⁵ studied the respiration and catalase activity in mosaic diseased sugarcane plants. In the present investigation, the effect of virus infection on catalase and peroxidase activity has been studied in 6 different varieties of sugarcane.

Leaves from healthy and mosaic infected plants of 6 sugarcane varieties, viz., B.O.11, B.O.32, B.O.47, Co. 527, Co. 1347 and Co. S. 416, were taken separately and analyzed for catalase and peroxidase activities. 4 samples were taken in each trial. Catalase activity was measured by the method of DEKOCK et al.⁶, with slight modifications. For enzyme preparation, 1 g of leaf tissue was crushed in a chilled mortar with 10 ml of phosphate buffer (pH 6.8) and a pinch of acid-washed sand. This homogenate was made up to 25 ml volume with glass-distilled water. A series of flasks containing 5 ml of 1.5% sodium borate and 1.5 ml of phosphate buffer (pH 6.8) was prepared. At zero time, 1 ml of homogenate was pipetted into each flask. The reaction was stopped in successive flasks after 1, 2, 3 and 4 min by rapidly adding 10 ml of $N H_2SO_4$. The remaining perborate was then titrated with 0.05 N $KMnO_4$ to the first pink colour which lasted for 30 sec.

Peroxidase activity was measured by the method given by PERUR⁷. Enzyme preparation was made by taking 1 g of sugarcane leaf and grinding it in a chilled mortar with 5 ml of glass-distilled water. The homogenate was made up to 50 ml and, after mixing well, it was filtered through cheese-cloth. This filtrate was the enzyme preparation which was used for the measurement of activity. In a test-tube, 10 ml of acetate buffer (pH 4.5) was taken. To it 1 ml of enzyme preparation and 0.5 ml of 1% pyrogallol were added. The contents were thoroughly mixed. At zero time, 0.5 ml of 0.05 N hydrogen peroxide was added and change in optical density was measured at 430 nm, using filter No. 43 in AIMIL Biochem. Absorptiometer (manufactured on Hilger pattern) at the end of 10 min. Data obtained are presented in Figures 1 and 2. The results showed that catalase activity was slightly weaker,

whereas the peroxidase remained slightly higher in diseased samples of all the 6 varieties.

It is customary to class catalase among the respiratory enzymes, although its real function is poorly known as yet². The activity of catalase generally decreases in virus infected plants^{4,8}. YAMAFUGI et al.⁵, in one of their experiments, found that catalase activity was weaker in the mosaic infected sugarcane. According to them, the

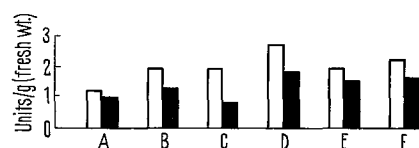


Fig. 1. Catalase activity of healthy (□) and mosaic diseased (■) samples.

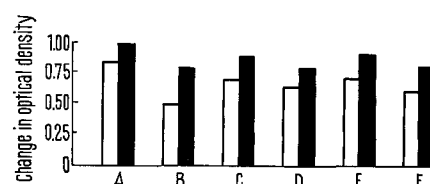


Fig. 2. Peroxidase activity of healthy and mosaic diseased samples. (A) B.O. 11; (B) B.O. 32; (C) B.O. 47; (D) Co. 527; (E) Co. 1347; (F) Co. S. 416. Dark-coloured rectangles show diseased samples and empty rectangles show healthy samples.

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