J. Indian bot. Soc. **60** : 154-159, 1981

CYTOLOGICAL EFFECTS OF TWO FUNGICIDES (CUPRAMAR, PHYGON-XL) ON *SPIROGYRA PARADOXA* RAO[1]

Y. S. R. K. SARMA AND P. ABHAYA VARDHANI

Laboratory of Algal Cytology and Cytogenetics, Department of Botany, Banaras Hindu University, Varanasi

ABSTRACT

The effects of two fungicides, namely, Cupramar and Phygon-XL were studied on the nuclear cytology of *Spirogyra paradoxa* Rao. Three different concentrations of Cupramar (10, 20 and 30 ppm) and Phygon-XL (0.1, 1.0 and 5 ppm) were applied for 1 and 2 h duration. In case of Cupramar concentrations of 50 ppm for 48 h and 100 ppm for 24 h proved to be sublethal and lethal doses respectively. Whereas in case of Phygon-XL, 10 ppm for 24 h and 50 ppm for 2 h proved to be sublethal and lethal doses respectively. The various nuclear and chromosomal aberrations as a result of treatment with different concentrations of the two fungicides were assessed qualitatively and quantitatively.

INTRODUCTION

Interest in the effects of pesticides has increased considerably with the knowledge that the organism may become resistant to application of these and that the use of pesticides has many secondary consequences. Such compounds have been of unquestionable economic importance but secondary effects produced by these compounds in which the hereditary constitution of the treated organisms may be altered are becoming apparent (Wuu and Grant, 1966). Fungicides have been in use in control of pathogenic fungi on plants of agricultural importance. These are less extensively used for the control of algae. Studies on the karyological effects of fungicides exist on higher plants (Kostoff, 1939 ; Wuu and Graut, 1966 ; Prasad and Cramer, 1968 ; Ramel, 1969 ; Ahmed *et al.*, 1972 and Bridges, 1975) and animals (Tezuka *et al.*, 1978). The effects of fungicides on eukaryotic algae from cytological viewpoint have not received due attention, although some work on algae has been carried out covering only physiological aspects (Zweig *et al.*, 1968). The present work, therefore, has been undertaken with a view to studying the effects of two fungicides namely, Cupramar (active ingradient copper oxychloride) and Phygon-XL (active ingradient 2,3-dichloro-1-4-napthoquinone) on the karyology of a filamentous green alga *Spirogyra paradoxa* Rao (Conjugales, Chlorophyceae).

MATERIAL AND METHODS

The material of *Spirogyra paradoxa* Rao was isolated as per standard methods (cf. Pringshiem, 1946) and was subsequently grown as clonal culture in modified Chu

1. Accepted for publication on August 19, 1980.

Thanks are due to Head, Department of Botany for providing laboratory facilities. Financial assistance in the form of SRF by CSIR, New Delhi to one of the authors (PAV) is gratefully acknowledged.

10 medium fortified with 8-10% garden soil extract. The original Chu 10 medium (cf. Chu, 1942) was modified in the present study by replacing $FeCl_3$ with Fe-EDTA. In addition to this, A_5 micronutrient solution (cf. Arnon, 1938) was also added. Cultures were then maintained in a culture chamber at 21 ±1°C under approximately 2500 Lux illustration for 16 h daily.

A few filaments of the actively growing material were treated in separate experiments with the fungicides Cupramar (Bharat Pulverising Mills Private Ltd., Bombay) with 10, 20 and 30 ppm for 1 and 2 h and Phygon-XL (United States Rubber, Naugatuck Chemical Division, Naugatuck, Connecticut, U.S.A.) with 0.1, 1.0 and 5 ppm for 1 and 2 h in each of the concentrations. Immediately after the said treatment period, a part of the, material was left in the chemical solution to see the viability of the alga in the chemical medium, and the remaining portions of the material were taken out and washed thoroughly three to four times with sterilized double distilled water so as to make it free of the chemical, and were then transferred to fresh nutrient medium for studying karyological effects. The materials were fixed at the time of maximum mitotic division during dark phase of the light-dark cycle up to seven days in Carnoy's fluid (1 : 3 glacial acetic acid and ethyl alcohol mixture). Cytological preparations were made following Godward's iron-alum acetocarmine technique (Godward, 1948) and were assessed on quantitative basis.

OBSERVATIONS

In case of Cupramar, concentration of 50 ppm for 48 h and 100 ppm for 24 h proved to be sublethal and lethal doses respectively, whereas in case of Phygon-XL, 10 ppm for 24 h and 50 ppm for 2 h proved to be sublethal and lethal doses respectively.

Various morphological and cytological abnormalities recorded in the affected cells (Figs. 1-8) with the various treatments of fungicides are considered under three categories : cytotoxic, mitotoxic and chromotoxic.

Cytotoxic :

1. Increase in size and number of pyrenoids in the chloroplasts of the treated materials particularly in lower concentrations than in the control.

2. Frequent degeneration of chloroplasts and fragmentation of filaments into few-celled bits in treatments with high concentrations.

3. Increase in the size of nucleus and nucleolus and occasional vacuolisation of either of these two or both.

4. formation of bi-, tri-, tetra- and pentanucleate cells due to the failure of daughter nuclei to move sufficiently apart or due to failure of septum-formation,

5. occasional appearance of enucleate cells.

Mitotoxic :

1. Diagonal to transverse orientation of nuclei with respect to cell axis, possibly due to disturbances in orientation of spindle.

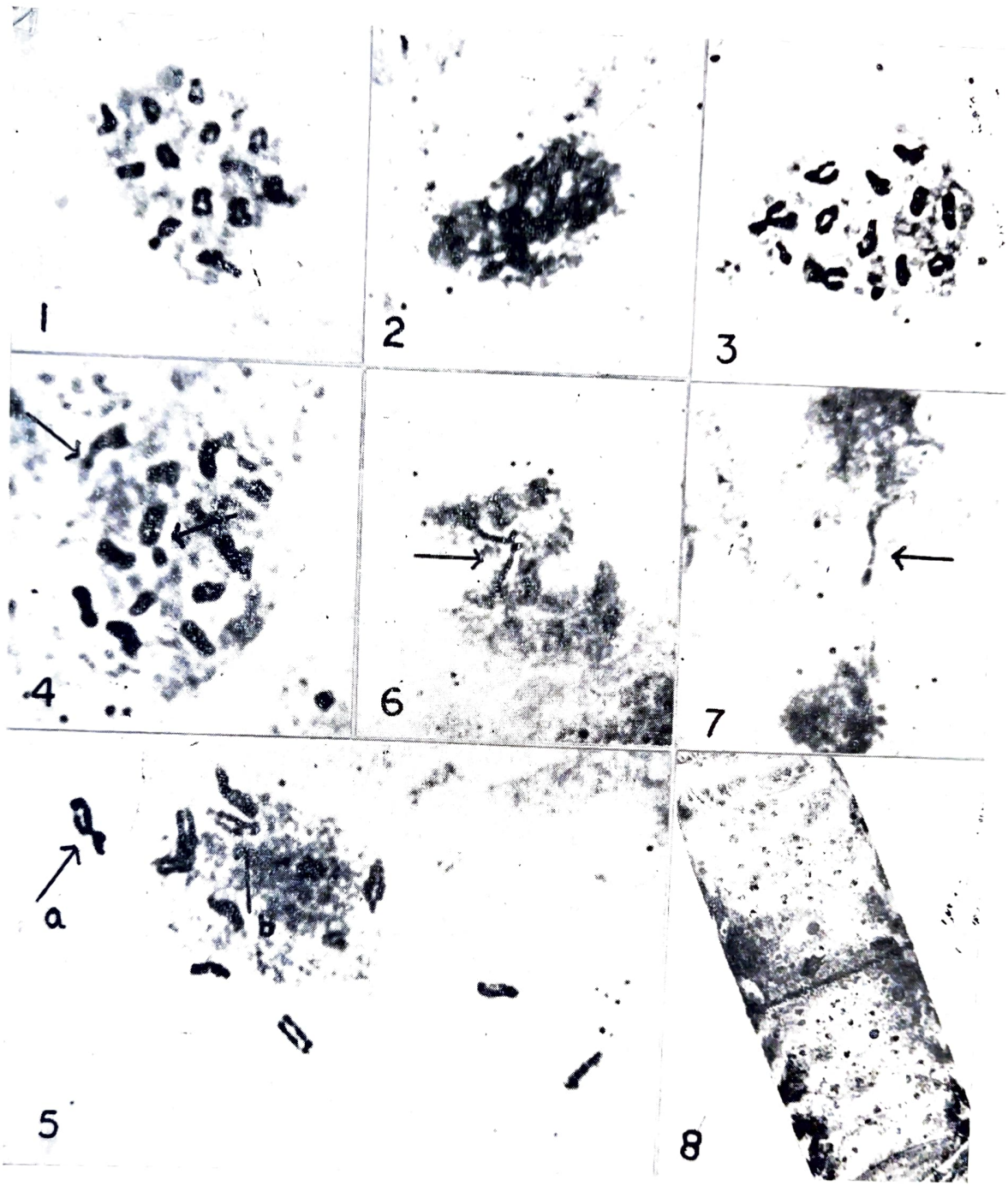
2. asynchronous division of the nuclei in bi- and trinucleate cells.

Chromotoxic :

1. Extreme condensation of chromosomes as compared with the control,

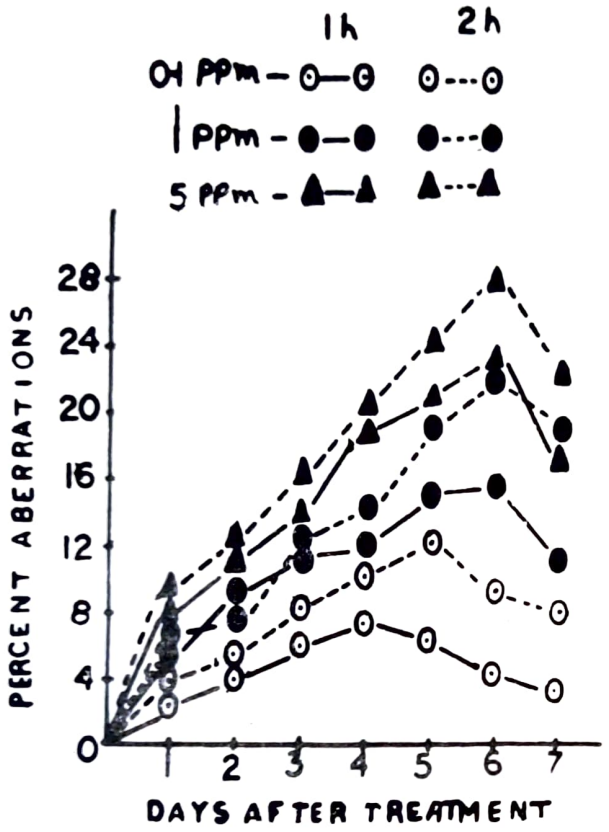
2. clumping of chromosomes,

3. frequent chromosome breaks adding up to two fragments resulting in up to $n=14$ ($n=12$ polycentric chromosomes in control material),



Figs × 1—8. *Spirogyra paradoxa.*

Fig. 1 Metaphase of control material showing n = 12 chromosomes. Fig. 2. Part of a cell showing vacuolisation of nucleus. Fig. 3. Metaphase showing n = 13 Chromosomes. Fig. 4. Metaphase showing chromosome breakage. Fig. 5. Prophase showing chromosome (a) and chromatid break (b). Fig. 6. Part of a cell showing anaphase bridge formation with daughter chromatids of a long chromosome. Fig. 7. Part of a cell showing laggard at anaphase. Fig. 8. Part of a filament showing transverse orientation of daughter nuclei and aenucleage cell. (Figs. 1—7 × 1800; 8 × 240)



Figs. 9. Percentage of aberrations at different concentrations of phygon-XL for 1 and 2 h duratiion.

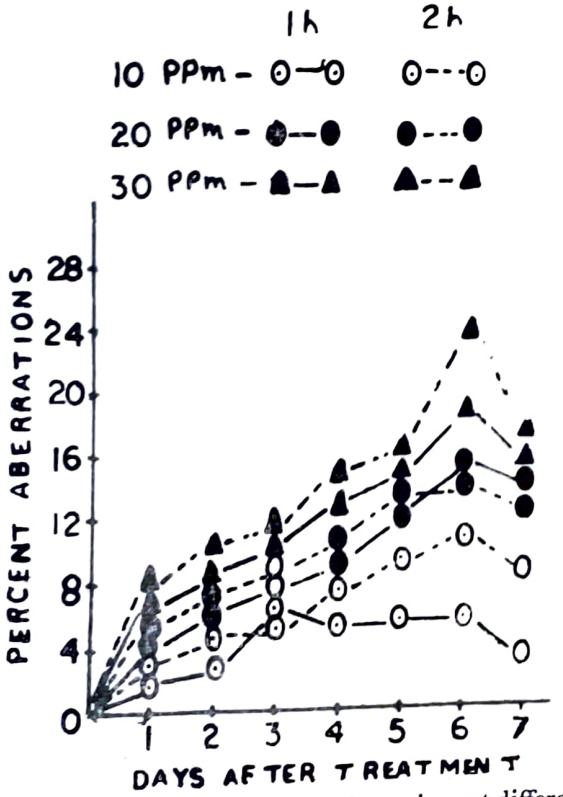


Fig. 10. Percentage of aberrations at different concentrations of Cupramar for 1 and 2 h duration.

4. occasional chromatid breaks,

5. lagging of long chromosomes in their anaphasic separation resulting in the formation of anaphase bridges,

6. occasional presence of laggards,

7. rare occurrence of micronuclei in a few cells.

Quantitative assessment of karyological effects was also made. Asynchronous division in bi- and trinucleate cells, breakage of chromosomes/chromatids, formation of bridges, laggards and micronuclei have been counted for the estimation of the percentage of cells with mitotic abnormalities. The percentage of cells with mitotic aberrations increased both with increase in the concentration of the chemical and duration of the treatment (Figs. 9 and 10). The percentage of aberrant cells were found to be maximum on 6th day, i.e. 28% with 5.0 ppm of Phygon-XL for 2 h and 24% with 30 ppm of Cupramar for 2 h. However, the percentage came down to 22 and 17 respectively on the seventh day. The material treated with 50 ppm of Cupramar for 4 h and 10 ppm of Phygon-XL for 4 h could not recover in the chemical-free medium even after 15 days and turned colourless.

DISCUSSION

In traces, copper is essential for the life processes of some algae (Walker, 1953). At increased concentrations, it acts as an algicide or an algostatic agent. Cupramar contains about 50% copper oxychloride. The algicidal effect of copper compounds have been reported by many workers. Fitzgerauld and Faust (1963) emphasized the importance of maintaining copper containing products in the immediate algal environment for prevention of algal growth. Faungstrom (1958) found an inhibitory effect of copper on photo-

synthesis of *Scendesmus quadricauda*, while a similar effect was recorded by Stemann Nielson *et al.* (1969) and Stemann Nielson and Kamp Nielson (1970) in *Chlorella pyrenoidosa* even with as low a concentration as 1 μg/ml.

Copper sulphate, an economical and selectively toxic algicide, has been extensively used to control undesirable growth in fresh water lakes and reservoirs. The significance of copper in the metabolism of *Chlorella* was first described by Green *et al.*, (1939), who investigated the inhibitory effect of Cu^{2+} chelating agents on respiration and photosynthesis of *Chlorella pyrenoidosa*. $CuSO_4$ at 0.1 to 20 ppm inhibited photosynthesis in *Lyngbya nigra* and at higher concentrations the effect was immediate (Gupta and Arora, 1978). Kanazawa and Kanazawa (1969) suggested that the membrane is the site of copper inhibition. 1 0 μg/ml of $CuSO_4$ in four days of contact, caused complete death of *Cladophora* (Betzer and Yehuda, 1969). The previous studies suggest that most algae are susceptible to 1-2 ppm of copper (Fitzgerauld *et al.*, 1952 and Maloney and Palmer, 1956). Some, however, tolerate much higher concentrations (Galloway and Krass, 1959). In the present study, somewhat higher concentrations, i.e. 100 ppm of Cupramar for 24 h proved to be lethal to the alga *Spirogyra paradoxa*.

Phygon-XL is a quinone fungicide. Furuta (1902) reported the algicidal effect of quinone. Later, Fitzgerauld *et al.*, (1952) recorded the selective toxicity of quinone compounds on some cyanophycean taxa. Phyton-XL, Cupramar and Unizeb at 0.001% (i.e. 100 ppm) were, found to be lethal to *Cephaleuros* (Jose and Chowdary, 1978). In the present study 50 ppm of Phygon-XL for 2 h was found to be lethal to *Spirogyra paradoxa*. There are also reports of some fungicides like Nabam, Captan and Lignosan which are more effective to check algal growth even at lower concentrations (Lazoroff, 1967 and Ukeles, 1962).

As far as the authors are aware, no studies on the karyological effects of fungicides on green algae exist, although Srivastava and Sarma (1978) reported such effects with two insecticides on *Oedogonium gunnii*, and hence the significance of the present investigation. However, there exist some reports regarding the karyological effects of some fungicides (not the same as used in the present study) on animal tissues and higher plants. The fungicide, Captan induced on a significant increase in the frequency of cells showing stickiness and severe inhibition of cell division at concentrations of 3.0 and 4.0 μg/ml in cultured human diploid cells (Tezuka *et al.*, 1978). Botran at 100, 200 and 300 ppm for 3, 6 and 12 h duration induced chromosome fragments and anaphase bridges in *Vicia faba* (Wuu and Grant, 1966). Ferbam one of the currently employed carbamate pesticides in agriculture at 1000 ppm for 30 and 60 min. induced unusually high percentage of chromosomal aberrations in *Allium cepa* (Prasad and Cramer, 1968). Panogan a mercurial fungicide induced C-mitosis at 1, 2 and 5 ppm for 1, 2 and 3 h in *Tradescantia* and *V. faba* (Ahmed and Grant, 1972). In the present investigation the various chromosomal aberrations have been recorded with 0.1, 1.0 and 5.0 ppm of Phygon-XL and 10, 20 and 30 ppm of Cupramar for 1 and 2 h duration.

From the present study it is evident that the fungicides are capable of producing various chromosomal aberrations in algae also. Regarding the efficacy of fungicides in inducing chromosomal aberrations and lethality between Cupramar

and Phygon-XL the latter was found to be more potent in the present study on *Spirogyra paradoxa*.

REFERENCES

AHMED, A. AND W. F., GRANT 1972. Cytological effects of the mercurial fungicide Panogan 15 on *Tradescantia* and *Vicia faba* root tips. *Muta. Res.* **14** : 391-396.

ARNON, D. I. 1938. Microelement in culture solution of experiments with higher plants. *Amer. J. Bot.* **25** : 322.

BETZER, N. AND Y., KOTT 1969. Effects of Halogens on algae—II *Cladophora* sp. *Water Research* **3** : 257-264.

BRIDGES, B. A. 1975. The mutagenesity of captans and related fungicides. *Mut. Res.* **32** : 3-34.

CHU, S. P. 1942. The influence of mineral composition of the medium on the growth of planktonic algae. (1) Methods and culture media. *J. Ecol.* **30** : 284-325.

FAUNGSTROM, I. 1972. The effects of some chelating agents and their copper complexes on photosynthesis in *Scenedesmus quadricauda*. *Physiol. Plant.* **27** : 389-397.

FITZGERAULD, G. P. AND S. L. FAUST 1963. Factors effecting algicidal and algistatic properties of copper. *Appl. Microbiol.* **11** : 345-351.

FITZGERAULD, G. P., G. C. GERLOFF, AND F. SKOOG, 1952. Studies on chemicals with selective toxicity to blue green algae. *Sewage Indian Waste.* **24**(7) : 888-896.

FURUTA, T. 1902. On the poisonous action of Quinone. *J. Coll. Agri. Tokyo Imp. Univ.* [*Bulletin* **4** : 407-410.

GALLOWAY, R. A. AND R. W. KRAUSS 1959. The differential action of chemical agents, specially polymyxin B, on certain algae, bacteria and fungi. *Am. J. Bot.* **46** : 40-49.

GODWARD, M. B. E. 1948. The iron-alum acetocarmine technique for algae. *Nature* **161** : 203.

GREEN, L. F., J. F. MCCARTHY AND C. G. KING 1939. Inhibition of respiration and photosynthesis in *Chlorella pyrenoidosa* by organic compounds that inhibit copper catalysis. *J. Biol. Chem.* **78** : 447-453.

GUPTA, A. B. AND A. ARORA 1978. Morphology and physiology of *Lyngbya nigra* with reference to copper toxicity. *Physiol. Plant.* **44** : 215-220.

JOTE, G. AND Y. B. K. CHOWDARY 1977. Effects of some Herbicides and Fungicides on the growth of the *Cephaleuros* in culture. Symposium on Phisology of Microorganisms. 119-134.

KANAZAWA, I. AND K. KANAZAWA 1969. Specific inhibitory effect of copper on cellular division in *Chlorella*. *Plant Cell Physiol.* **10** : 495-502.

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

LAZOROFF, N. 1967. Algae response to pesticide pollutants. *Bacteriol. Proc.* No. G 149, 48.

MALONEY, T. E. AND C. M. PALMER 1956. Toxicity of six chemical comounds in thiry cultures of algae. *Water Sewage Works* **103** : 509-513.

PRINGSHIEM, E. G. 1946. Pure cultures of algae, their preparation and maintenance. Cambridge, England, University Press.

PRASAD, J. AND D. CRAMER 1968. Genetic effects of Ferbam on *Asperigillus niger* and *Allium cepa*. *Phytopathology* **58** : 1188-1189.

RAMEL, C. 1969. Genetic effects of organic compounds. 1. Cytological investigations on *Allium* roots. *Hereditas* **61** : 208.

STEMANN NIELSEN, E. AND L. KAMP NIELSEN 1970. Influence of deleterious concentrations of copper on the growth af *Chlorella pyrenoidosa*. *Physiol. Plant.* **23** : 828-840.

STEMANN NIELSEN AND S. WIUM-ANDERSEN 1969. The effect of deleterious concentrations of copper on the photosynthesis of *Chlorella pyrenoidosa*. *Physiol. Plant.* **22** : 1121-1133.

SRIVASTAVA, S. AND Y. S. R. K. SARMA 1978. Effects of two insecticides Dimecron and Nuvan on the survival, growth and nuclear cytology of *Oedogonium gunnii* With. *J. Cytol. and Genet.* (in press).

TEZUKA, H., S., TERAMOTO, M. KANEDA, R. HENNER, N., MURAKAMI AND Y., SHIRASU 1978. Cytogenetic and dolinant lethal studies on captan. *Mut. Res.* **57** : 201-207.

UKELES, R. 1962. Growth of pure cultures of marine phytoplankton in the presence of toxicants. *Appl. Microbiol.* **10** : 532-537.

WALKER, J. B. 1953. Inorganic micronutrient requirements of *Chlorella*. I. Requirements for calcium (or strontium), copper and molybdenum. *Arch. Biochem. Biophys.* **46** : 1-11.

WUU, K. D. AND W. F. GRANT 1966. Chromosomal aberrations induced in somatic cells of *Vicia faba* by pesticides. *The Nucleus* **10**(1) : 37-46.

ZWEIG, G., J. E. HILT AND R. MC MAHON 1968. Effect of certain quinones diquat and diuron on *Chlorella plumulosa*. *Weed Science* **16** : 69-73.