Nematol. medit. (1991), *19*: 155-162

Istituto di Nematologia Agraria, C.N.R. — 70126 Bari, Italy

EFFECT OF PARAQUAT ON THE SUPEROXIDE DISMUTASE AND CATALASE ACTIVITY OF TOMATO PLANTS SUSCEPTIBLE AND RESISTANT TO *MELOIDOGYNE INCOGNITA*

by

G. ZACHEO, G. GUIDA, BLEVE-ZACHEO and M.T. MELILLO

Summary. Paraquat applied to tomato seedlings as aqueous solution or to tomato root culture incorporated in agar Gamborg's medium, affected the plant growth. Seedlings of the cultivars Roma VF and VFN8, susceptible and resistant to the root-knot nematode *Meloidogyne incognita*, respectively, wilted when supplied with more than 10 μM paraquat (PQ). Tomato roots grown in a medium containing more than 5 μM PQ ceased growing and became necrotic. The activities of two related enzymes, superoxide dismutase and catalase, were followed in seedlings and cultured tomato roots treated with different PQ concentrations. Paraquat caused a pronounced increase in the activity of superoxide dismutase and catalase. The changes in enzymatic activity appeared to result from enzyme activation which was an adaptive response to provide protection against the herbicide.

Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride, methyl viologen) (PQ) is a widely used herbicide, that is metabolized in various cells to yield the paraquat monocation radical. Paraquat radicals react very fast with oxygen to generate the superoxide radical (O_2^-) (Hassan and Fridovich, 1978). Paraquat also causes a rapid and pronounced increase in the rate of biosynthesis of the manganese-containing superoxide dismutase and a moderate induction of catalase activity in *Escherichia coli* (Hassan and Fridovich, 1977). It seems likely that paraquat subverts a portion of the normal, cyanide-sensitive, electron flow in the cells and transfers that electron flow to oxygen in a univalent cyanide-insensitive manner. This results in an increased rate of production of O_2^-, or some product uniquely derived from O_2^-. The radicals induce or derepress the biosynthesis of the manganese-superoxide dismutase, which defends the cell against their deleterious reactivities (Hassan and Fridovich, 1977). A similar defense mechanism has been shown in the incompatible plant-pathogen interaction (Doke *et al.*, 1987; Zacheo and Bleve-Zacheo, 1988). Intact potato leaves responded to infection with *Phytophthora infestans* by generating O_2^- before cellular penetration regardless of race of the pathogen. Further activation of O_2^- generation followed penetration by incompatible, but not compatible, races. In the cells of aged, wounded tuber tissues, O_2^- generation was also found to be activated after penetration of cells by incompatible, but not compatible, races. The O_2^- generation increased as the number of penetrated cells increased and was followed by hypersensitive cell death (Doke *et al.*, 1987). We have also demonstrated that nematode infection increased the rate of cyanide-resistant respiration and induced the O_2^- generation in resistant tomato cultivars (Zacheo and Bleve- Zacheo, 1987; Zacheo and Molinari, 1987; Molinari *et al.*, 1990). In susceptible tomato plants the production of superoxide anions was scavenged by the increased superoxide dismutase activity and in so doing provided an important defense against oxygen toxicity. Paraquat in procaryotes induced a subversion of the electron flow from the normal water-producing pathway to one with O_2^- and H_2O_2 production. We postulated that the toxic chemicals of paraquat might induce a traumatic effect in the tissues and influence the synthesis of enzymes such as SOD, catalase, peroxidase etc. in tomato plants. For this reason we undertook an investigation of the mechanism of paraquat-induced toxicity in two tomato root cultivars, susceptible and resistant to *Meloidogyne incognita*, with particular regard to the importance of the level of superoxide dismutase and catalase in protecting the root tissue against the oxidative challenge produced by paraquat.

Materials and methods

Seeds of tomato (*Lycopersicon esculentum* Mill.) cvs Roma VF susceptible and VFN8 resistant to *M. incognita* were soaked in absolute ethanol for 2 min and sterilized for 10 min in a 1.5% solution of NaOCl. They were then washed in sterile distilled water and transferred to petri dishes containing Gamborg's basal medium (Sigma). When the seeds had germinated, 1 cm length root tips

were excised and transferred to plastic petri dishes containing Gamborg's basal medium supplemented with 0-5 μM PQ. Some dishes were inoculated with *M. incognita* race 2 juveniles and were used for histological studies.

Seedlings of both tomato cultivars were germinated in sterilized quartz sand. Uniformly germinated seeds were transferred into 3 cm clay pots containing quartz sand, exposed to 0-50 μM of PQ concentration and divided into two groups, one uninfested and used as the control, and the other immediately inoculated with active juveniles of *M. incognita* race 2. Measurements were made on seedlings 5 days after inoculation with nematodes.

The level of superoxides (O_2^-) was measured in terms of reduced NBT (formazan) (Wenning *et al.*, 1975). The complete reaction mixture was 10 mM phosphate buffer pH 7.8, 1 mM EDTA, 20 μM NADPH, 0.05% NBT and 0.2 g (fresh weight) of roots in a total volume of 2 ml. Washed roots were put into the complete reaction mixture without NBT and maintained under vacuum for 5 min. The reaction mixture, plus roots, was then exposed to the atmosphere (oxygen) and NBT was added; 30 min later the mixture containing the roots was heated at 85°C for 15 min and cooled. The reduced NBT was calculated from OD at 580 nm and the reducing activity of NBT was expressed as increase of OD_{580} per hour per gram dry weight.

Five days after nematode inoculation, root tissues or rooted seedlings were homogenized in 100 mM EPPS buffer, pH 8.5, in a Potter homogenizer cooled in ice. The resultant slurry was filtered through 4 layers of cheesecloth and centrifuged at 10,000g for 20 min. The supernatant fraction was used for enzymatic assays and proteins. The supernatant was centrifuged at 131,000g for 1 hr to yield a pellet of microsomal membranes which was then used to form a membrane suspension (2 mg protein/ml in 2 mM EPPS, pH 8.5)(Mayak *et al.*, 1983). The microsomes and the supernatant remaining after centrifugation (soluble fraction) were used for enzymatic assay. SOD was assayed spectrophotometrically at 25°C according to the procedure described by McCord and Fridovich (1969) modified by Furusawa *et al.* (1984). The reaction was performed in a1-ml cuvette containing 50 mM phosphate buffer, pH 7.8, 1 mM EDTA, 20 μM cytochrome c (Sigma type VI), 0.1 mM xanthine and 12.5 μg of xanthine oxidase (Boerhinger, Mannheim). Reduction of cytochrome c was followed at 550 nm with reference at 540 nm using a Perkin-Elmer 557 dual-wavelength spectrophotometer. One unit of SOD is defined as the amount of enzyme which inhibits the reduction rate of cytochrome c by 50%. Unit of enzyme activity was proportional to V/v — 1 where V and v are the reduction rates of cytochrome c in the absence and in the presence of SOD, respectively.

Catalase assays were perfomed with an oxygen monitor equipped with a Clark electrode. Aliquots of tissue homogenate were used to initiate the catalase reaction with 15 mM H_2O_2 in 50 mM, pH 7.0 K-Pi buffer. Reactions were run at 25°C (Olsen and Cook, 1987). Protein was determined according to Lowry *et al.* (1951).

For histological observations infested or galled roots were fixed in 3% glutaraldehyde in 0.05 M cacodylate buffer pH 7.2 for 6 hours at 4°C, rinsed several times in the same buffer and post- fixed in 2% osmium tetroxide for 4 hours at 4°C. The roots were dehydrated in an ethanol series and embedded in Spurr's medium (Spurr, 1969). Sections 2 μm thick were stained with toluidine blue and examined with a light microscope.

All experiments were repeated four times. Values are expressed as means ± standard errors.

Results

The tomato roots of the VFN8 and Roma VF cultivars were exposed to a wide range of PQ concentrations in Gamborg's agar medium. Growth rates were completely inhibited by concentrations of 10 μM.At 0.5 μM paraquat decreased the growth rate by 30% and at 5 μM by 90% in both cultivars (Fig. 1). Exposure of tomato roots to PQ in petri dishes exposed to light caused an induction of the superoxide dismutase. This induction of the O_2^- scavenging enzymes is thought to represent an important defense

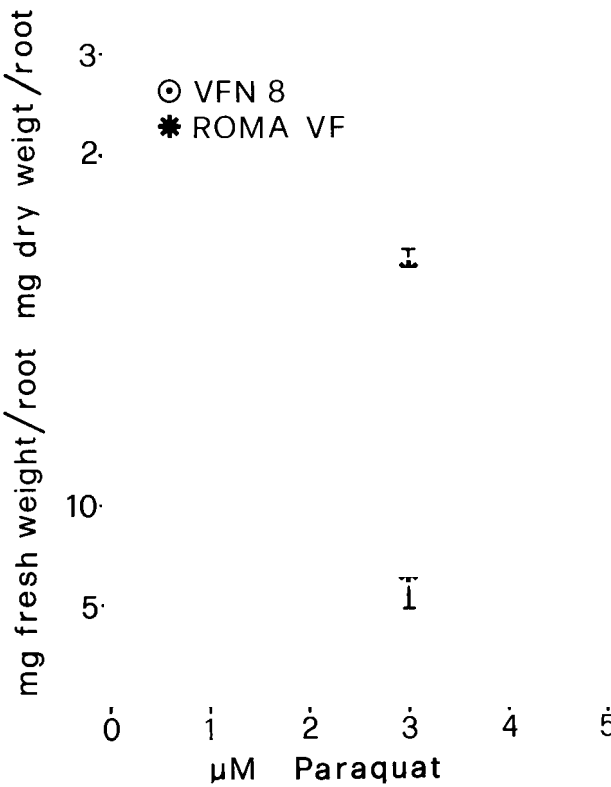


Fig. 1 - Effects of paraquat on tomato root growth. Roots were cultured in Gamborg's medium containing PQ for 5 days. Tissue was dried at 70°C for 24 h.

against PQ toxicity (Moody and Hassan, 1982). Fig. 1 presents the level of superoxide dismutase present in VFN8 and Roma VF roots grown in Gamborg's medium over a range of PQ concentrations. The roots in both cultivars responded to PQ by increasing their content of superoxide dismutase. The VFN8 roots, however, were not able to increase their level of superoxide dismutase to match the increased flux of O_2^- caused by increasing the concentration of PQ beyond 3 μM. The roots of the cultivar Roma VF continued to increase their superoxide dismutase level and were protected from PQ toxicity. Paraquat caused induction of SOD biosynthesis in different cellular fractions. As shown in Fig. 2, the SOD activity increased in both soluble and microsomial fractions. Furthermore, as the enhanced activity of SOD led to an increase in the production of H_2O_2, it might be expected that there would also be an increase in the biosynthesis of catalase. Fig. 3 presents the results of testing these possibilities. The roots of Roma V and VFN8 grown in the presence of 0.5-5 μM paraquat showed a striking increase in catalase. However, roots of cultivar Roma VF showed a greater increase of this enzyme than the cultivar VFN8 in all the fractions examined. At 5 μM PQ, total catalase activity increased 1.7 fold in Roma VF and 1.3 fold in VFN8.

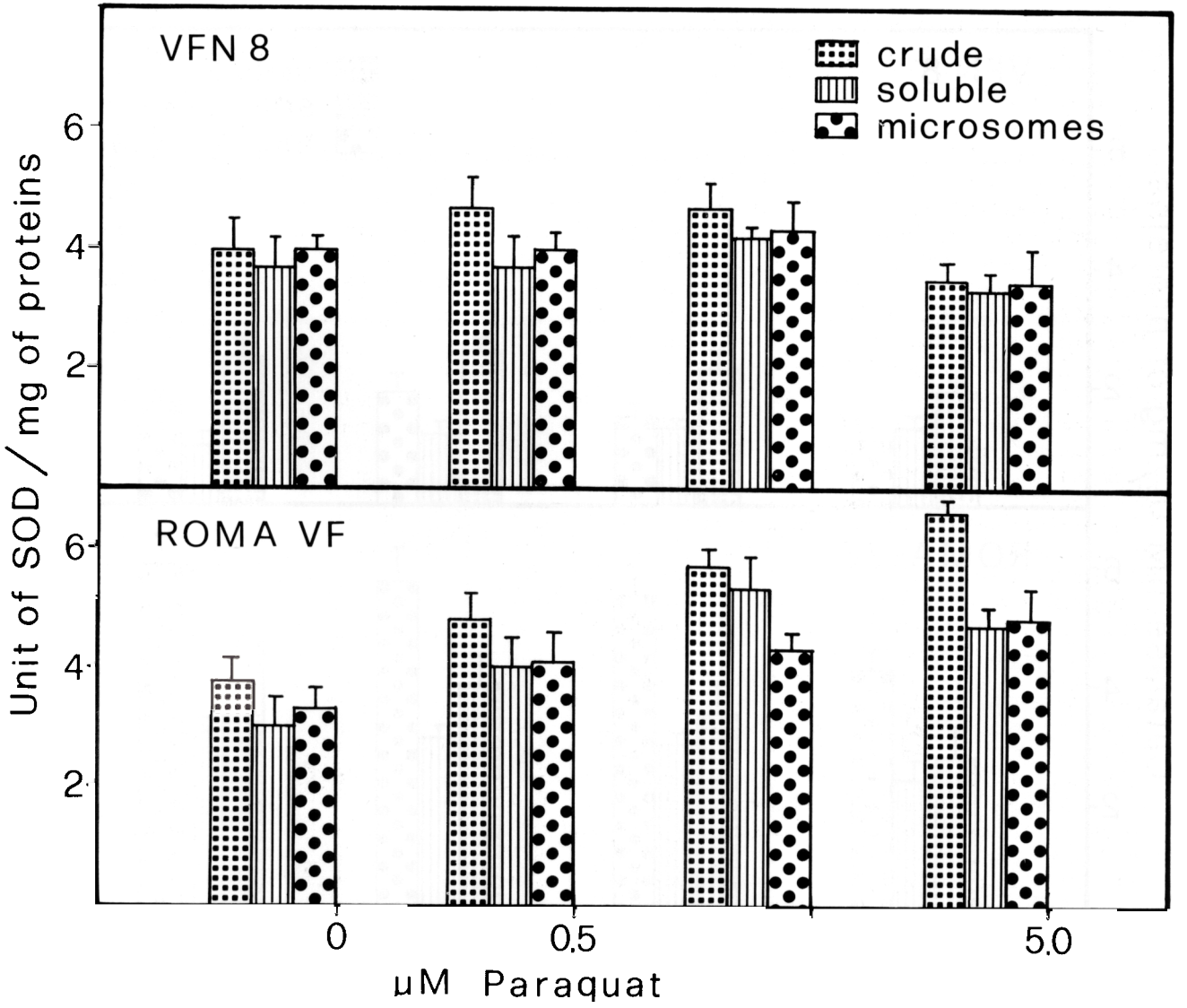
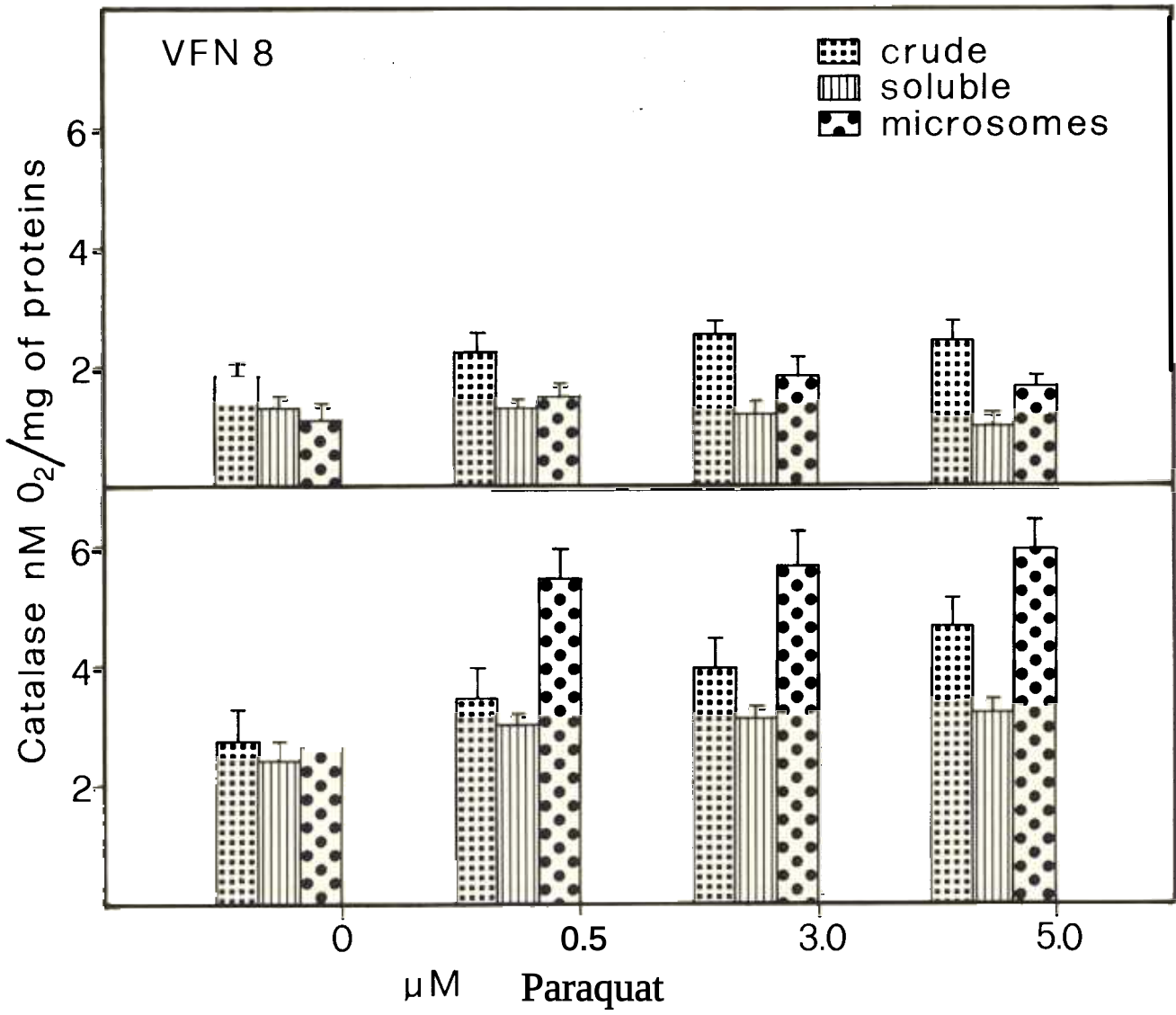


Fig. 2 - Paraquat effects on SOD content of tomato root cultures. Roots grown in absence and in presence of paraquat were analyzed for superoxide dismutase activity in three different fractions (crude, soluble, microsomial membranes). Analytical values were given as units/mg/protein ± SE.

Paraquat (0.5-5 μM) incorporated in the axenic solid medium did not influence the physiology of nematode and gall formation in the Roma VF roots. Multinucleate cells in which the nematode fed, were observed in the galls, but the paraquat treatment induced dramatic changes in the neighbouring tissue. Galled tissue after PQ treatment became necrotic and this phenomenon, which could be a form of senescence, increased markedly at the higher PQ concentrations (Fig. 4). At 0.5 μM PQ the cortical cells were collapsed and some were necrotized (Fig. 3b); 3 μM PQ induced a necrotic zone involving partly cortical cells and extending to the endodermis, pericycle and vascular bundles (Fig. 3b, c); at 5 μM PQ callus tissue was observed near the vascular cylinder which pressed the giant cells together and they became necrotic. The nematode juveniles seldom got sufficient nutrients and thus failed to complete their development.

When paraquat is metabolized in the cells it yields the PQ monocation radical. This radical is reoxidized to produce O_2^- intracellularly (Hassan and Fridovich, 1978). The rate of PQ mediated O_2^- generation was determined by measuring *in vivo* the reduction of NBT in the tomato roots (Table I). In both Roma VF and VFN8 incubated in the presence of 0-50 μM PQ the NBT induction (O_2^- generation) level decreased. At 5 μM PQ the roots of Roma VF showed a greater rate of decrease (60%) of O_2^- produc-



tion than the roots of VFN8 (30%); at 10 μM the O_2^- production in Roma VF was more or less blocked whereas the level of superoxides remained unchanged in VFN8 roots. At 50 μM PQ the production of O_2^- in VFN8 was blocked. This high reduction of superoxides could be due to an increase in SOD activity. As shown in Tables' II and III the units of superoxide dismutase per mg of extractable proteins increased in the presence of 0.5-10 μM PQ; the increase was less remarkable in VFN8. In tomato roots grown in the presence of PQ and infected with *M. incognita* juveniles the SOD activity increased when compared with the uninfected roots and reached the maximum in Roma VF treated with 10 μM PQ. At 50 μM PQ, there was a general decline in proteins and fresh weight content (Tables II and III) and a decrease in SOD activity was observed. Expressed on the basis of protein content, the activity of catalase increased during PQ treatment in both the cultivars whether infected or uninfected (Tables II and III). The addition of 0-0.5 μM PQ to Roma VF resulted in a linear increase of catalase activity. Since the activity of catalase increased sharply, it is clear that paraquat caused a disproportionate increase in the rate of biosynthesis of this enzyme, in comparison to other cell proteins. A similar trend, but less remarkable, was detected in VFN8 cultivar.

TABLE I - *Superoxide dismutase production (NBT reduction) by roots of tomato cv Roma VF and VFN8. Seedlings were grown in presence of 0-50 μM paraquat. NBT reduction is reported as OD 580 per g of dry weight (d.w.).*

Treatment μM PQ	Reduced NBT OD 580/g of d.w.	
	Roma VF	VFN8
0	148 ± 20	229 ± 20
5 μM	60 ± 13	165 ± 16
10 μM	16 ± 2	167 ± 20
50 μM	14 ± 2	19 ± 3

Discussion

Tomato plants wilted when treated with a paraquat solution of more than 50 μM concentration *in vivo*. Growth of tomato roots *in vitro* was inhibited even at 3 μM PQ and nematode development was inhibited even in susceptible Roma VF because of the necrosis of tissues induced by PQ. Necrotised cells surrounded the giant cells in the susceptible roots similarly to that described for the resistant ones when an hypersensitive reaction takes place.

With regard to the biological significance of the reactions described in this paper, the key question is whether PQ may provide a mechanism for superoxide production and how it may cause an induction of superoxide dismu-

TABLE II - *The effect of paraquat on growth and enzymatic activities in seedlings uninfected (Uninf.) and infected (Infect.) with* M. incognita *of tomato cv VFN8 (resistant). Seedlings were watered with different concentrations of paraquat (0-50 μM). All values are means ± SE of four experiments.*

Treatment μM PQ	Fresh Weight		Proteins (mg/g.f.w.)		SOD U/mg of proteins		Catalase nM O_2/min/mg of pr.	
	Uninf.	Infect.	Uninf.	Infect.	Uninf.	Infect.	Uninf.	Infect
0	2.86 ± 0.31	3.31 ± 0.18	6.32 ± 0.51	4.50 ± 0.10	3.62 ± 0.10	3.89 ± 0.31	1.98 ± 0.10	1.79 ± 0.11
5	3.51 ± 0.20	3.80 ± 0.26	4.61 ± 0.49	3.82 ± 0.32	3.81 ± 0.15	4.00 ± 0.27	2.45 ± 0.12	2.25 ± 0.16
10	2.82 ± 0.19	2.52 ± 0.30	2.97 ± 0.31	3.11 ± 0.10	4.04 ± 0.10	4.08 ± 0.25	2.68 ± 0.15	2.41 ± 0.17
50	1.38 ± 0.41	1.16 ± 0.32	3.01 ± 0.21	3.20 ± 0.22	1.82 ± 0.30	0.31 ± 0.50	2.92 ± 0.10	2.90 ± 0.31

TABLE III - *Seedlings of Roma VF tomato cv (susceptible) treated as in Table II. Each value is mean ± SE of four experiments.*

Treatment μM PQ	Fresh Weight		Proteins (mg/g.f.w.)		SOD U/mg of proteins		Catalase nM O_2/min/mg of pr.	
	Uninf.	Infect.	Uninf.	Infect.	Uninf.	Infect.	Uninf.	Infect.
0	2.91 ± 0.21	3.12 ± 0.41	6.30 ± 0.71	4.51 ± 0.26	3.52 ± 0.45	5.12 ± 0.13	2.18 ± 0.45	3.65 ± 0.21
5	3.10 ± 0.13	2,58 ± 0.18	4.62 ± 0.36	3.80 ± 0.16	4.21 ± 0.31	5.62 ± 0.41	3.45 ± 0.26	3.98 ± 0.36
10	1.59 ± 0.31	1.23 ± 0.17	2.97 ± 0.24	3.31 ± 0.21	5.36 ± 0.20	6.56 ± 0.32	4.01 ± 0.18	5.02 ± 0.50
50	1.10 ± 0.40	1.01 ± 0.21	3.01 ± 0.31	3.25 ± 0.31	2.02 ± 0.31	1.82 ± 0.51	4.35 ± 0.26	5.22 ± 0.30

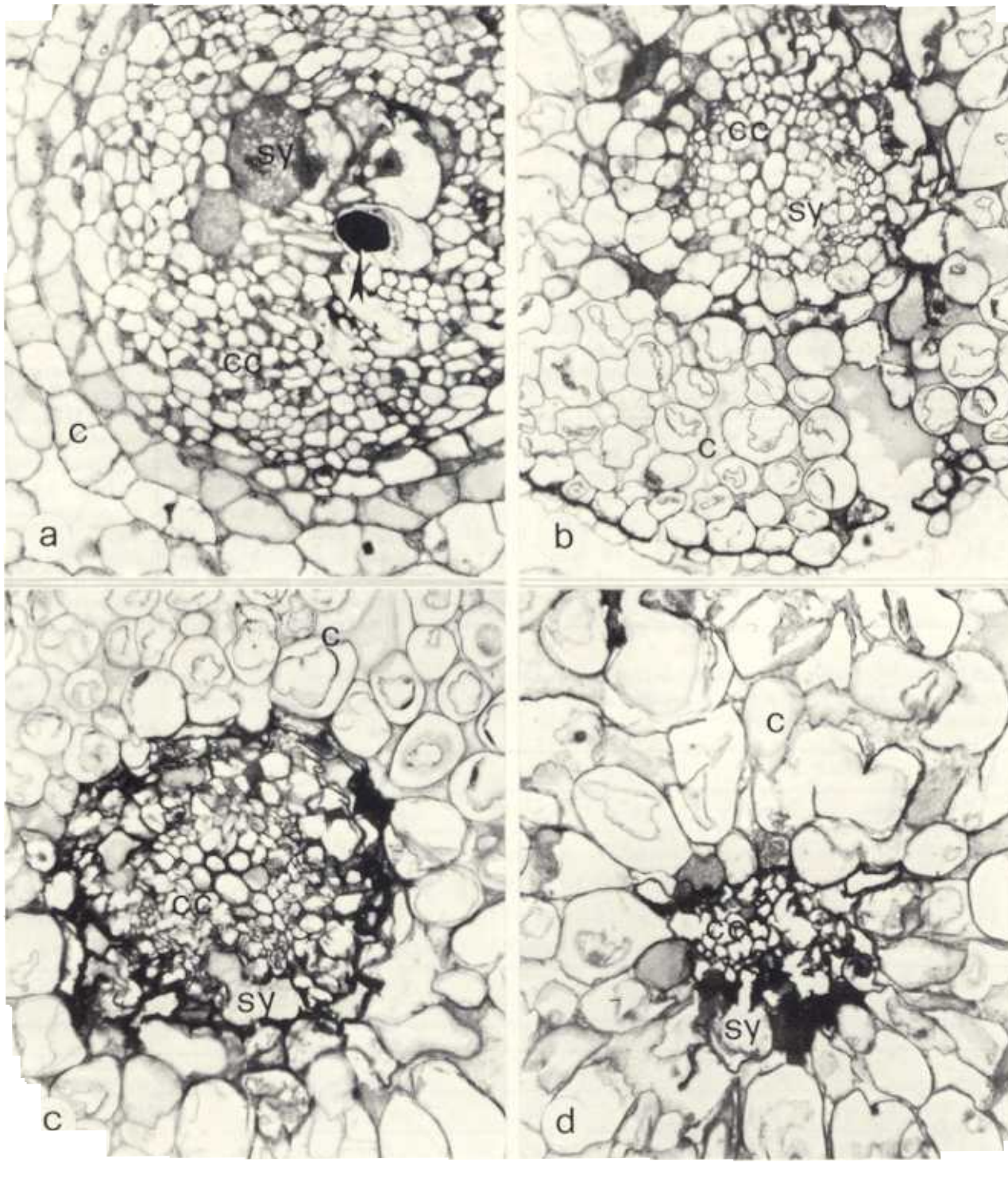


Fig. 4 - a): Micrograph of a transverse section through a syncytium in a root of cv Roma VF grown in Gamborg's medium ten days after nematode (arrow) inoculation. b), c), d): Transverse sections of ten day infected roots grown in presence of 0.5, 3 and 5 μM paraquat respectively. sy = syncytium, c = cortical cells, cc = central cylinder. x 850.

tase and catalase in tomato root tissues. In both cultivars included in the present study paraquat induced increased activity of SOD and catalase. This is consistent with earlier results (Nakano and Asada, 1980). The application of paraquat results in the increased photoreduction of molecular oxygen which produces the superoxide anion radical (Farrington *et al.*, 1973). The conditions which increase the production of superoxides in cells are similar to those that induce the biosynthesis of SOD and catalase in bacteria (Hassan and Fridovich, 1977), animals (Stevens and Autor, 1977) and plants (Rabinowitch *et al.*, 1983). Furusawa *et al.* (1984) demonstrated that for the protection of the cells of tobacco callus against the superoxide and other active of oxygen species derived from superoxide, an increased SOD activity is required. In plants, SOD content varies depending on their age (Tanaka and Sugahana, 1980) and senescence is inhibited by maintaining high levels of SOD and catalase (Dhindsa *et al.*, 1982). Thus, it appears that the biosynthesis of SOD is regulated by the concentration of superoxides in cells (Tanaka *et al.*, 1988).

The present study showed that PQ toxicity and increased activities of SOD in tomato plants are related phenomena. However, the induction of superoxide dismutase by paraquat was lower in the resistant cultivar. This finding suggests that the detoxifying mechanism in tomato roots of VFN8 was depressed or not sufficiently activated. It appears that plants resistant to *M. incognita* have a reduced genetic capability to scavenge superoxide anions. Therefore the hypersensitive reaction induced by the nematode in the resistant tomato roots is largely due to the ability of the tissue to increase the intracellular production of O_2^- (Zacheo and Bleve-Zacheo, 1988). The increase in SOD content, induced by paraquat, was more dramatic in Roma VF and could be due to the activation of some precursor polypeptide rather than to *de novo* synthesis, because paraquat decreased the protein content of the root tissues. Thus the induction of external flux of O_2^- by paraquat, should increase the SOD activity. In turn, the increased SOD activity would lead to a large production of H_2O_2 and catalase. We can deduce that the increased production of O_2^-, caused by paraquat, is an important factor in the toxicity of this herbicide and the increased activity of different enzymes (*i.e.*, superoxide dismutase, catalase, antioxidants) is a defence against this aspect of its toxicity. As proposed by Keppler and Novacky (1987) the superoxide radicals generated by paraquat induced peroxidation of lipids which produces an increase in membrane permeability and electrolyte leakage. The results presented here are consistent with this fact. The cortical cells (Fig. 4) of tomato root galls induced by *M. incognita* were completely collapsed when exposed for 10 days to 0.5-5 μM PQ. Therefore, it is possible that exposure of plants to low concentrations of paraquat for short periods (2-4 days) may produce superoxide radicals at the plasma membrane level and their release into the cell. The production of superoxide radicals inside the cell could produce a biochemical signal to increase the level or activity of superoxide dismutase and catalase. When plants were treated with higher concentrations (50 μM *in vivo* or 10 μM *in vitro*) they suddenly wilted and became senescent. As a consequence a decrease in superoxide dismutase was observed.

In conclusion, SOD and catalase activities could determine the abundance of superoxides and hydrogen peroxide in tissues stressed by chemicals. Thus a decreased activity of SOD and catalase could result in an elevated steady state level of O_2^-, H_2O_2, $OH^{\cdot}$ and O_2^*. This would, in turn, result in membrane deterioration, decreased ability to retain solutes, necrosis and death of plant tissues.

The authors thank Mr. R. Lerario for skillful technical assistance.

Literature cited

DHINDSA R.S., PLUMB-DHINDSA P.L. and REID D.M., 1982 - Leaf senescence and lipid peroxidation: effects of some phytohormones, and scavengers of free radicals and singlet oxygen. *Physiol. Plant., 56*: 453-457.

DOKE N., CHAI H.B. and KAWAGUCHI A., 1987 - Biochemical basis of triggering and suppression of hypersensitive cell response. *In*: Molecular Determinants of Plant Diseases (S. Nishimura, C.P. Vance and N. Doke, Eds.), pp. 235-251. Springer- Verlag, Berlin.

FARRINGTON J.A., EBERT M., LAND E.J. and FLETCHER K., 1973 - Bipyridylium quaternary salts and related compounds. V. Pulse radiolysis studies on the reaction of paraquat radical with oxygen. Implication for the mode of action of bipyridyl herbicides. *Biochem. Biophys. Acta, 314*: 372-385.

FURUSAWA T., TANAKA K., THANUTONG P., MIZUGUCHI A., YAZAKI M. and ASADA K., 1984 - Paraquat resistant tobacco calluses with enhanced superoxide dismutase activity. *Plant and Cell Physiol., 25*: 1247-1254.

HASSAN M.M. and FRIDOVICH I., 1977 - Regulation of the synthesis of superoxide dismutase in *Escherichia coli*. Induction by methyl viologen. *J. Biol. Chem., 252*: 7667-7672.

HASSAN M.M. and FRIDOVICH I., 1978 - Superoxide radical and the oxygen enhancement of the toxicity of paraquat in *Escherichia coli*. *J. Biol. Chem., 253*: 8143-8148.

KEPPLER L.D. and NOVACKY A., 1987 - The initiation of membrane lipid peroxidation during bacteria-induced hypersensitive reaction. *Physiol. and Molec. Plant Pathol., 30*: 233-245.

LOWRY H., ROSEBROUGH N.J., FARR A.L. and RANDALL R.J., 1951 - Protein measurement with the Folin phenol reagent. *J. Biol. Chem., 193*: 265-275.

MAYAK S., LEGGE R.L. and THOMPSON J.E., 1983 - Superoxide radical production by microsomal membranes from senescing carnation flowers: an effect on membrane fluidity. *Phytochemistry, 22*: 1375-1380.

MCCORD J. and FRIDOVICH I., 1969 - Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem., 244*: 6049-6055.

MOLINARI S., ZACHEO G. and BLEVE-ZACHEO T., 1990 - Effects of nematode infestation on mitochondria isolated from susceptible and resistant tomato roots. *Physiol. and Molec. Plant Pathol., 7*: 27-37.

MOODY C.S. and HASSAN H.M., 1984 - Mutagenicity of oxygen free radicals. *J. Biol. Chem., 259*: 12821-12825.

NAKANO Y. and ASADA K., 1980 - Spinach chloroplasts scavenge hydrogen peroxide on illumination. *Plant Cell Physiol., 21*: 1295-1307.

OLSEN J.A. and COOK C.R., 1987 - Superoxide dismutase in

wounded etiolated pea seedlings. *Phytochemistry, 26*: 71- 73.
RABINOWITCH H.D., CLARE D.A., CRAPO J.D. and FRIDOVICH I., 1983 - Positive correlation between superoxide dismutase and resistance to paraquat toxicity in the green alga *Chlorella sorokiniana. Biochem. Biophys. Acta, 225*: 640-648.
SPURR A.R., 1969 - A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrast. Res., 26*: 31-43.
STEVENS J.B. and AUTOR A.P., 1977 - Induction of superoxide dismutase by oxygen in neonatal rat lung. *J. Biol. Chem., 252*: 3509-3514.
TANAKA K. and SUGAHARA K., 1980 - Role of superoxide dismutase in defense against SO_2 toxicity and an increase in superoxide dismutase activity with SO_2 fumigation. *Plant and Cell Physiol., 21*: 601-611.
TANAKA K., FURUSAWA I., KONDO N. and TANAKA K., 1988 - SO_2 tolerance of tobacco plants regenerated from paraquat- tolerant callus. *Plant Cell Physiol., 29*: 743-746.
WENNING R.S., WEBER R. and ROOS D., 1975 - Quantitative aspects of the producing of superoxide radicals by phagocytizing human granulocyte. *J. Lab. and Clin. Medic., 85*: 242-245.
ZACHEO G. and BLEVE-ZACHEO T., 1987 - Stimulation of respiratory pathways in tomato roots infested by *Meloidogyne incognita. Physiol. and Molec. Plant Pathol., 30*: 461-466.
ZACHEO G. and BLEVE-ZACHEO T., 1988 - Involvement of superoxide dismutases and superoxide radicals in the susceptibility and resistance of tomato plants to *Meloidogyne incognita* attack. *Physiol. and Molec. Plant Pathol., 32*: 313-322.
ZACHEO G. and MOLINARI S., 1987 - Relation between root respiration and seedlings age in tomato cultivars infested by *Meloidogyne incognita. Ann. appl. Biol., 111*: 589-595.

Accepted for publication on 26 March