Nematol. medit. (1987), *15*: 139-144.

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

THE EFFECT OF A NEMATICIDE ON THE ASCORBATE SYSTEM DURING MATURATION OF TOMATO FRUITS IN THE FIELD

by
G. Zacheo, M. Di Vito and F. Tenaglia

It is reported that ascorbic acid is widespread in relatively high concentrations in plant and animal tissues (Chatterjee, 1973; Edgar, 1970). The application of large doses of ascorbic acid have been beneficial in detoxifying the excess hystamine produced or released in stress conditions (Chatterjee, 1973) including several viral diseases e.g. the common cold (Lewin, 1976). Primates and guinea-pigs which lack the capacity to synthesize ascorbic acid, are susceptible to metabolic defects associated with scurvy if deprived of this in their diet (Lewin, 1976).

High levels of ascorbic acid are present in maturing fruits and during their storage, and give protection against infection by bacterial pathogens (Ogata *et al.*, 1975; Van Lelyveld, 1975). Ascorbic acid is generally considered to provide resistance in plants to various pathogens (Farkas *et al.*, 1960; Maine and Kelman, 1961). Low levels of ascorbic acid in tomato cultivars and pea breeding lines were associated with their susceptibility to nematode attack (Arrigoni *et al.*, 1979; Zacheo *et al.*, 1981).

The investigation reported here was concerned with changes in the ascorbate system in the roots and fruits of tomato, grown in the field and treated or untreated with a fumigant nematicide, and exposed to infestation by *Meloidogyne javanica* (Treub) Chitw.

Materials and methods

The experiment was made in a field at Ginosa Marina (Province of Taranto) infested with 15 eggs and/or juveniles/ml soil of *M. javanica*.

Sixteen plots (1.25×3.50 m) were each treated with 500 l/ha of Di-Trapex (80% 1,3 dichloropropene 1,2 dichloropropane+20% methyl isothiocyanate) applied with a hand injector one month before transplanting. The same number of plots were left untreated. Ten tomato plants were planted per plot; there were two inbred lines of the Institute of Nematology of Bari «84214» and «85143», resistant to root-knot nematodes, the resistant hybrid cv. «Zenith» and the susceptible cv. «Ventura». Each treatment was replicated 4 times and the experiment was arranged in a randomized block design. During the experiment the plants were irrigated, fertilized and protected against diseases and pests. The root galling index of all plants was evaluated at the end of the experiment using a 0-5 scale (Di Vito *et al.*, 1979).

Roots or fruits for the determination of ascorbic acid and enzyme activities were washed thorougly in distilled water and dried with filter paper. Five grams of roots or 100 grams of fruits were homogenized in a solution of 5% metaphosphoric acid and centrifuged at 25,000 g for 15 min; 0.1 ml of this extract was added to 2.9 ml of 0.1 M citrate 0.2 M phosphate buffer at pH 6.2, in a quartz cuvett and the optical density (OD) at 265 nm was determined with a UV-visible spectrophotometer (ACTA C III, Beckman Instruments Inc.). The ascorbic acid was oxidized by adding a small quantity of ascorbic acid oxidase (Boehringer) and the relative decrease in OD was determined again (Dawson and Magee, 1955). Concentrations of ascorbic acid were expressed as μg/mg of proteins and compared with a standard curve (10-100 μg) of pure ascorbic acid.

Roots (20 g) or tomato fruits (100 g) were homogenized with 100 mM potassium phosphate, pH 7.8, containing 1 mM EDTA in a glass potter cooled in ice. The homogenate was squeezed through 4 layers of gauze and centrifuged at 12,000 g for 30 min. The resulting precipitate was resuspended and dialysed against 20 mM phosphate buffer pH 7.8. The dialysed material was centrifuged at 10,000 g for 10 min and the supernatant was used for the determination of enzyme activity and proteins.

AFR-reductase activity was assayed by measuring the rate of NADH oxidation at 340 nm in the presence of AFR generating system (Arrigoni *et al.*, 1981). The reaction mixture contained 0.2 mM NADH enzyme (400 μg protein), 1 mM AA and 1 U AA-oxidase.

Ascorbic acid oxidase activity was measured by following the disappearance of ascorbic acid at 265 nm (Maxwell and Bateman, 1967). The sample cuvett contained 1.0 ml 0.2 M sodium phosphate buffer at pH 6.2, 0.2 ml 0.001 M ascorbic acid, 0.2 ml enzyme extract, and distilled

water to bring the cuvett contents to 3.0 ml. Proteins were determined by the method of protein-dye binding (Bradford, 1976).

Results

The AFR-reductase activity, measured in the extracts from the roots, differed significantly between the nematode susceptible cv. Ventura and the resistant cv. Zenith and the two inbred lines (Table I).

Soil treatment with the fumigant nematicide had no effect on the enzyme activity in the root extracts, which in each case was comparable between treated and untreated plots (Table I).

Ascorbate oxidase activity was more or less at the same level in all the root extracts from all of the plants in the untreated soil. A significant increase in the enzyme activity, however, was present in roots from cv. Ventura grown in fumigated soil compared with the level of activity in non-fumigated soil (Table I).

In the unripe (green) fruits, the ascorbic acid content was high in all of the plants and decreased as the fruits matured (Table II). The ascorbic acid content of the unripe fruit from plots treated with the nematicide was increased considerably compared with untreated plots. There were no significant differences in the ascorbic acid content of mature fruits from treated or untreated plots (Table II). Unripe fruit from the two inbred lines and cv. Zenith had similar levels of AFR-reductase activity which, however, was increased by nematicide treatment of the soil.

Table I - *Activity of AFR-reductase and ascorbate oxidase in roots of tomato lines and cultivars grown in* Meloidogyne javanica *infested soil treated with Di-Trapex.*

Tested tomato plants	Galling index		AFR-reductase (a)		Ascorbate oxidase (b)	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
Line 84214	0	0	7.4±0.5	6.8±0.8	49±2	46±2
Line 85143	0	0	11.2±0.4	15.4±1.2	44±2	49±4
Cv. «Zenith»	0	0	13.0±1.0	8.1±0.9	48±1	53±2
Cv. Ventura	4.5±0.2	2.0±0.3	3.1±0.2	3.0±0.6	42±2	70±3

(a) - AFR-reductase activity expressed as μg NADH/min×10 mg proteins;
(b) - ascorbate oxidase activity expressed as μg ascorbic acid/min×mg proteins.

There was a relatively higher level of enzyme activity in unripe fruit from cv. Ventura but this was not increased by nematicide treatment. There was a decrease in the AFR-reductase activity in the mature fruits, particularly in cv. Ventura, but this was not significantly influenced by the nematicide treatment (Table III).

Ascorbate oxidase enzyme was not present in any of the tomato fruits.

Discussion

Higher levels of AFR-reductase were evident in roots of resistant tomatoes compared with the susceptible cv. Ventura.

These data confirm previous studies which showed that ascorbic acid and products of its oxidation are factors involved in the mechanism of biological resistance in plants (Arrigoni *et al.*, 1979; Zacheo *et al.*, 1981) Ascorbic acid is transformed, through the ascorbate oxidase activity, into ascorbic free radicals (AFR) as intermediate compounds, and then into dehydroascorbic acid as a final product. Because of the high level of AFR-reductase activity in roots of resistant tomatoes, AFR is reconverted in large quantities of ascorbic acid and consequently the utilization of this product is very high.

The low level of AFR-reductase activity in the susceptible cv. Ventura renders it unable to reconvert the AFR to ascorbic acid. As a consequence the quantity of ascorbic acid which the susceptible plant can utilize, is lower than in the resistant plants. The oxidation of ascorbic acid in the susceptible cv. Ventura is also related to the increased activity of the ascorbate oxidase enzyme, which further decreases the ascorbic acid content in the root tissues.

The ascorbic acid content increased in the unripe fruits from all the plants grown in nematicide-treated plots. The treatment possibly stimulated the biosynthetic activity of the plants so that the increased activity of AFR-reductase led to high levels of ascorbic acid in the fruits. Because of the absence of ascorbate oxidase enzyme in the fruits, the combination of the two factors may account for the higher content of ascorbic acid in the unripe fruits.

The ascorbic acid content decreased in ripening fruits in all the plants, which is in accordance with data reported by Van Lelyveld (1975) and by Ogata *et al.*, (1975). These authors reported a decline in the ascorbic acid content in mango fruits and okuras during maturation and storage. Treatment of the soil with a nematicide is considered to protect plants by

Table II - *Ascorbic acid content in unripe and mature fruits of tomato plants grown in* Meloidogyne javanica *infested soil treated with Di-Trapex.*

Tested tomato plants	Unripe fruits		Mature fruits	
	Untreated	Treated	Untreated	Treated
Line 84214	14.9 ± 0.7	19.7 ± 0.8	3.2 ± 0.1	3.9 ± 0.1
Line 85143	28.7 ± 1.4	41.6 ± 1.2	3.4 ± 0.1	3.9 ± 0.2
Cv. «Zenith»	34.9 ± 2.3	55.0 ± 2.9	3.8 ± 0.2	4.4 ± 0.1
CV. Ventura	38.5 ± 1.2	39.1 ± 1.3	3.5 ± 0.3	3.1 ± 0.3

Table III - *AFR-reductase activity in unripe and mature fruits of tomato plants grown in* Meloidogyne javanica *infested soil treated with Di-Trapex.*

Tested tomato plants	Unripe fruits		Mature fruits	
	Untreated	Treated	Untreated	Treated
Line 84214	23.1 ± 1.4 (a)	32.2 ± 1.3	18.5 ± 1.4	24.8 ± 1.4
Line 85143	26.9 ± 1.4	35.2 ± 1.6	12.5 ± 0.2	14.1 ± 0.7
Cv. «Zenith»	28.1 ± 1.0	37.5 ± 2.6	11.6 ± 0.5	15.8 ± 0.7
CV. Ventura	34.0 ± 1.5	35.1 ± 1.7	9.3 ± 0.5	11.2 ± 0.5

(a) - AFR-reductase expressed as μg NADH/min × 10 mg proteins.

killing the nematode parasites as indicated in our results, but it may have the effect of inducing the stimulation of AFR-reductase activity in the fruits, which is expressed in the increased level of ascorbic acid.

SUMMARY

Higher levels of ascorbate free radical (AFR) - reductase were found in the roots of the nematode resistant cv. Zenith and two inbred lines compared with the susceptible cv. Ventura. Soil treatment with the nematicide Di-Trapex had no effect on the activity of AFR-reductase. There was an increase in ascorbic acid content in unripe fruits from all the plants grown in nematicide - treated plots. The nematicide treatment possibly stimulated the biosynthetic activity of the plants and, consequently, of the AFR-reductase which led to the high levels of ascorbic acid in the fruits.

LITERATURE CITED

ARRIGONI O., ZACHEO G., ARRIGONI-LISO R., BLEVE-ZACHEO T. and LAMBERTI F., 1979. Relationship between ascorbic acid and resistance in tomato plants to *Meloidogyne incognita*. *Phytopathology, 69*: 579-581.

BRADFORD M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. *Anal. Biochem., 72*: 248-254.

CHATTERJEE B.I., 1973. Evolution and the biosynthesis of ascorbic acid. *Science*, 182: 1271-1272.

DAWSON C.R. and MAGEE R.J., 1955, Ascorbic Acid Oxidase. *In*: Methods of Enzymology. Vol. II, Acad. Press, London, New York, S. Francisco, pp. 831-835.

DI VITO M., LAMBERTI F. and CARELLA A., 1979. La resistenza del pomodoro nei confronti dei nematodi galligeni: prospettive e possibilità. *Rivista di Agronomia, 13*: 313-322.

EDGAR J.A., 1970. Dehydroascorbic acid and cell division. *Nature, 227*: 24-26.

FARKAS G.L., KIRALY Z. and SOLYMOSY F., 1960. Role of oxidative metabolism in the localization of plant viruses. *Virology, 12*: 408-421.

LEWIN S., 1976. Vitamin C: Its Molecular Biology and Medical Potential. Acad. Press, New York, pp. 231.

MAINE E.C. and KELMAN A., 1961. The influence of reducing substances on resistance in bacterial wilt in tobacco. *Phytopathology, 51*: 491-492.

MAXWELL D.P. and BATEMAN D.F., 1967. Changes in the activities of some oxidases in extracts of *Rhizoctonia*-infected bean hypocotyls in relation to lesion maturation. *Phytopathology, 57*: 132-136.

OGATA K., YAMAUCHI N. and MINAMIDE T., 1975. Physiological and chemical studies on ascorbic acid of fruits and vegetables. I. Changes of ascorbic acid content during maturation and storage period in Okuras. *J. Jap. Soc. Hortic. Sci., 44*: 192-196.

VAN LELYVELD L.J., 1975. Ascorbic acid content and enzyme activities during maturation of the mango fruit and their association with bacterial black spot. *Agroplantae, 7*: 51-54.

ZACHEO G., ARRIGONI-LISO R., BLEVE-ZACHEO T., LAMBERTI F., PERRINO P. and ARRIGONI O., 1981. Changes of ascorbate free radical reductase in pea roots infested by *Heterodera goettingiana*. *Nematol. medit., 9*: 181-187.

Accepted for publication on 26 February 1987.