Relationship Between Ascorbic Acid and Resistance in Tomato Plants to *Meloidogyne incognita*

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ABSTRACT

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The role of ascorbic acid in the defence mechanism of tomato (*Lycopersicon esculentum*) plants against attack by the root-knot nematode, *Meloidogyne incognita*, was studied under controlled conditions. The ascorbic acid concentration in roots of either nematode-resistant or -susceptible plants was varied experimentally and the reaction of these plants to attacks by the nematode was tested. A decrease in ascorbic acid, obtained by application of lycorine (an inhibitor of ascorbic acid synthesis), induces a reduction of plant resistance to the nematode, but an artificial increase in ascorbic acid concentration transforms susceptible plants into resistant ones. The amount of ascorbic acid in susceptible plants was unaltered by nematode attacks but in resistant plants ascorbic acid synthesis always was stimulated. It is suggested that ascorbic acid is utilized for the synthesis of mitochondrial hydroxyproline proteins which control the development of cyanide-resistant respiration.

Although there are many publications on the presence of ascorbic acid in plant tissues, its role in plants has yet to be clarified. The content of ascorbic acid in plants varies from organ to organ, but it is usually high and in excess of the amount required for the respiratory processes of the cell. In fact, of the total oxygen consumed by a plant cell, only 3–4% is mediated by ascorbic oxidase and polyphenol oxidase; the rest is mediated via cytochrome oxidase (13,15). Recently it was demonstrated that ascorbic acid is utilized in large amounts in cell metabolism (3) and that it is needed to carry out in vivo synthesis of hydroxyproline-containing proteins in plants (5).

We suggest that ascorbic acid, through control of the biosynthesis of hydroxyproline-containing proteins, is involved in two biological processes: plant growth (14,17) and biological mechanisms of defence which are similar in both plants and animals (2,5,6). Following wounding of or a pathogen attack upon plant tissue, a metabolic event occurs which resembles that found in polymorphonuclear leucocytes of animals and which is of fundamental importance in phagocytosis (8): the development of cyanide-resistant respiration. The fact that ascorbic acid is needed for the development of cyanide-resistant respiration and that, within certain limits, the intensity of this respiration is directly correlated with the cell content of ascorbic acid (4), indicates that this compound is one of the factors involved in the mechanism of resistance in plants. The purpose of the present study was to ascertain if experimental variation of ascorbic acid in tomato (*Lycopersicon esculentum* Mill.) would modify plant resistance to the root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitw.

MATERIALS AND METHODS

Seeds of tomato cultivars Roma VF and Marmande (susceptible) and Rossol, VFN 8, Piersol, and Brech (resistant) were sown in steam-sterilized soil; and 6 to 7 cm tall, seedlings were transplanted to steam-sterilized sandy loam in 10-cm diameter plastic pots. The pots were placed in a growth chamber (27 C, 65% RH, and 3,000 lux for 15 hr each day). The resistant plants were irrigated daily with aqueous 0.1 mM lycorine (3,6), an alkaloid extracted from *Sternbergia lutea* Ker-Gawl (1), and the susceptible ones with an aqueous solution of ascorbic acid at different concentrations. Control plants received only tap water.

Approximately 1,000 active juveniles of *M. incognita* were pipetted into four equidistant holes spaced around the plants in each pot. There were six plants in each treatment.

To assess penetration by the nematode, roots were stained with lactophenol and acid fuchsin. Ascorbic acid was determined after roots were washed with tap water, rinsed thoroughly in distilled water, and dried. Five grams of roots 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, pH 6.2, in a quartz cuvette and the OD at 280 nm was determined with a UV-visible spectrophotometer ACTA C III (Beckman Instruments Inc., Fullerton, CA). The ascorbic acid was oxidized by adding a small quantity of ascorbic acid oxidase extracted from the exocarp of fresh marrow squash (*Cucurbita pepo* var. *italica* Tod.) (9), and the relative decrease in OD was determined again. Concentrations of ascorbic acid were expressed as μg/g of dry weight and compared with a standard curve (10–100 μg) of pure ascorbic acid. The results represent the mean of three experiments.

RESULTS

The application of 0.1 mM lycorine, an inhibitor of ascorbic acid synthesis (3,6), lowered the quantity of endogenous ascorbic acid in the four tomato cultivars resistant to *M. incognita* and altered their reaction to nematode attacks (Table 1). The greatest effect was observed in cultivar Brech in which there were more than twice as many larvae in lycorine-treated than in untreated plants.

TABLE 1. Populations of *Meloidogyne incognita* juveniles in roots of resistant tomato plants treated with 0.1 mM lycorine[a]

Tomato cultivar	Nematodes per gram root: Treated with 0.1 mM lycorine	Nematodes per gram root: Untreated	Increase with respect to untreated (%)
Rossol	369 **[b]	281	31.3
VFN 8	120 *	85	41.2
Piersol	651 ***	349	86.5
Brech	398 ***	186	114.5

[a]Lycorine was applied to plants 6 days before nematode inoculation. Nematode counts were made 24 days later. No effect on the viability of the nematode was observed after lycorine application: nematodes are unable to synthesize ascorbic acid (12).

[b]Statistical significance between treated and untreated was determined according to the Student's t-test: ***, **, *, significant at $P = 0.001$, 0.01, and 0.05, respectively.

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However, in all the other cultivars lycorine decreased the degree of resistance to root-knot nematode. Different response of the resistant plants to nematodes after treatment with lycorine might be attributed either to a different permeability of the root cells to the alkaloid or to a different capability for ascorbic acid synthesis.

Conversely, when the endogenous amount of ascorbic acid was artificially increased (16), tomato plants of the cultivar Roma, highly susceptible to *M. incognita,* had double the concentration of ascorbic acid (1,264 μg/g dry wt) in roots compared with untreated control seedlings (646 μg/g dry wt) after 5 days (Fig. 1). In both susceptible cultivars, Roma and Marmande, that were treated with ascorbic acid, the rate of nematode invasion was decreased with respect to untreated controls (Table 2); the higher the concentration of ascorbic acid used, the lower the root infection. The rate of root penetration by the nematode was reduced slightly at 5 mM ascorbic acid and was almost nil at 45 mM (Table 2). The reduced invasion of *M. incognita* resulted in a moderate degree of root galling and number of adults. Nevertheless, pretreatment of nematodes alone with ascorbic acid did not seem to affect their development in roots. The viability of the nematodes was not affected by ascorbic acid, since juveniles of *M. incognita* kept for 3 days in 35 mM invaded the roots as well as did untreated nematodes.

Lycorine and ascorbic acid, at the highest concentrations tested, slightly inhibited plant growth. Uninfected Brech plants treated with the alkaloid showed a root growth reduction of 22% (dry weight) compared with untreated plants, and Roma roots treated with ascorbic acid weighed 16% less than untreated ones. Nevertheless, the inhibitory effect that both these compounds have on plant growth cannot be correlated with their influence on root invasion by nematodes. In fact, ascorbic acid reduces infection almost to nil, while lycorine stimulates it remarkably.

To ascertain whether nematode invasion influenced ascorbic acid synthesis, concentrations of that compound were determined in roots of a susceptible (Roma) and a resistant (Rossol) cultivar at 6-day intervals after inoculation with *M. incognita*. The concentration of ascorbic acid in the roots of resistant plants increased gradually up to about 12 days after nematode inoculum was introduced, after which it returned to its initial level (Fig. 2). Conversely, the concentration of ascorbic acid in the roots of susceptible plants was not affected by nematode attack. A very gradual reduction of the compound was observed, which probably can be attributed to a tentative initiation of a defence reaction at the beginning of the attack (Fig. 2).

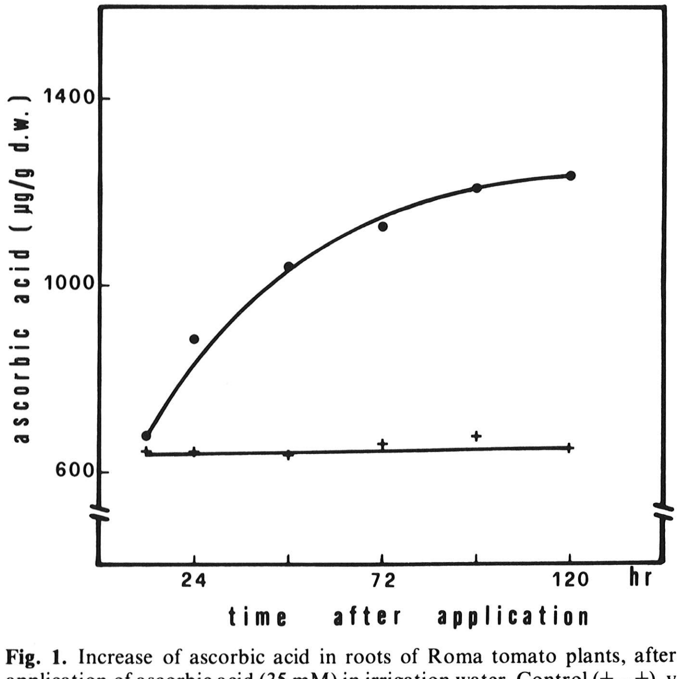

Fig. 1. Increase of ascorbic acid in roots of Roma tomato plants, after application of ascorbic acid (35 mM) in irrigation water. Control (+—+), $y = 635 + 0.341\ x + 0.001\ x^2$; treated (●—●), $y = 640 + 10\ x - 0.049\ x^2$.

DISCUSSION

Previous studies indicated that ascorbic acid is a factor involved in the mechanism of biological resistance in plants. Tonzig and Bracci (16) showed that different reactions of various organs of Leguminosae infected by *Rhizobium leguminosarum* are correlated with the content of ascorbic acid. Nodules generally are produced on roots which have a low content of ascorbic acid and rarely on leaves or stems, where the concentration of the acid is much higher. These authors also demonstrated that the application of this compound inhibited the formation of root nodules in *Pisum sativum* L. (16). Farkas et al (10) have reported that feeding ascorbic acid to *Nicotiana glutinosa* L. in high concentrations markedly inhibits lesion formation by tobacco mosaic virus. The same authors also showed that the amount of ascorbic acid consumed increased greatly in leaves exhibiting local lesions. Van Lelyveld (18,19) reported that mango fruits infected with bacteria, and showing lesions, have a lower ascorbic acid content than healthy fruits. A decrease in ascorbic acid content also was found in mosaic-affected spinach leaves (11).

Results of the present investigation confirm that ascorbic acid plays an important role in host-parasite interactions. Applications of this compound to susceptible plants inhibit nematode invasion and, conversely, a decrease in its endogenous content in resistant cultivars leads to an increase of nematode penetration. Our results indicate that it is not the endogenous level of ascorbic acid that differentiates susceptible and resistant plants, but the biosynthetic

TABLE 2. Populations of *Meloidogyne incognita* juveniles in roots of two susceptible tomato cultivars treated with ascorbic acid[a]

Concentration of ascorbic acid	Roma VF: Nematodes per gram of root (no.)	Roma VF: Decrease with respect to untreated (%)	Marmande: Nematodes per gram of root (no.)	Marmande: Decrease with respect to untreated (%)
Control	487		683	
5 mM	398	16.2	593	11.7
15 mM	294	39.6	421	38.4
35 mM	94	80.7	120	82.4
45 mM	17	96.5	4	99.4
LSD $P = 0.05$	85		140	
LSD $P = 0.01$	156		255	

[a]Ascorbic acid was applied to plants 6 days before nematode inoculation and nematode counts were made 24 days later.

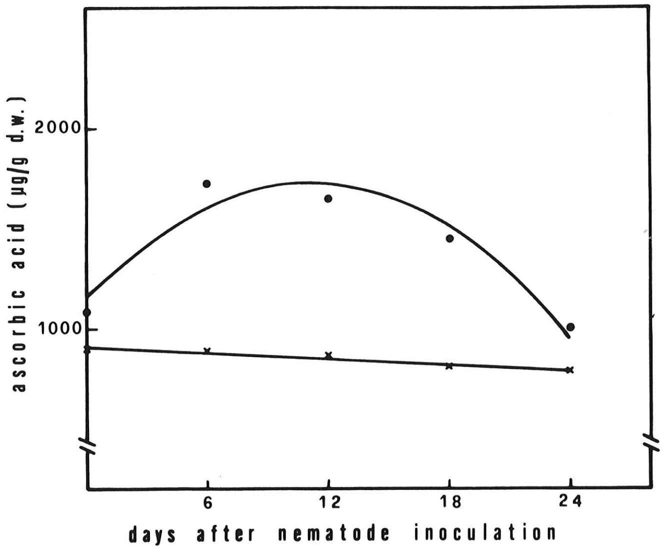

Fig. 2. Effect of attack of *Meloidogyne incognita* on the ascorbic acid concentration in roots of resistant and susceptible tomato cultivars. Susceptible (cultivar Roma): $y = 903 - 1.84\ x - 0.14\ x^2$ (X—X); resistant (cultivar Rossol): $y = 1{,}144 + 105\ x - 4.71\ x^2$ (●—●).

capability of the plant after parasite attack. In fact, the resistant cultivars synthesize large quantities of ascorbic acid after attack but the susceptible ones are unable to do this.

We suggest that ascorbic acid is mostly utilized for the synthesis of mitochondrial hydroxyproline proteins which control the cyanide-resistant respiration (2,7). This kind of respiration seems to develop earlier and to a larger extent in the resistant cultivars. This would explain the failure of a quick defence response in susceptible plants when nematode attack occurs. Our hypothesis is supported by other data which clearly show that nematode invasion induces an increase of mitochondrial hydroxyproline proteins in resistant cultivars but not in susceptible ones (20).

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