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Root-Knot Nematodes and the Process of Ageing in Plants

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Abstract: Infection of plants by root-knot nematodes is often accompanied by physiological changes characteristic of ageing. Ultra-low tissue luminescence of infected plants indicated oxidation of cell-membrane lipids. Cells with membranes subjected to oxidation lose some of their capacity for water retention. Treating tomato and radish with lidocaine hydrochloride, an inhibitor of lipid oxidation, retarded above-ground symptoms of root-knot nematode infection and of ageing. *Key Words: Meloidogyne hapla, Meloidogyne incognita,* antioxidant, lidocaine, luminescence.

It was noticed long ago that plants infected with root-knot nematodes differ from healthy plants by having drooping leaves that often begin to turn yellow earlier. Analyses of such leaves will almost invariably show that they contain a lower percentage of water by weight. Other changes noted (5) are also similar to those in ageing plants. The drooping of leaves is usually explained by impaired absorption of water from soil by the damaged root system, thus leading to a water deficit. Nevertheless, the classical test for water movement in the xylem, which involves immersion of the roots in a colored solution, or the standard manometric test for root pressure, will show that there is little, if any, difference in water transportability between healthy and Meloidogyne-infected plants, as long as no decay accompanies the infection.

Water metabolism can be disturbed by the oxidation of cell-membrane phospholipids (7). Such oxidation is usually inhibited by a complex anti-oxidative system. An important role in this system in animal organisms is thought to be played by vitamin E. Oxidation is not inhibited when there is malfunction of this vitamin, as in an old organism (2) or in cancerous tumors (4). As a result of this malfunction the phospholipids in the cell membranes are oxidised and form "hypophobic tails" (7). When that happens, cells retain water less well, lose turgor, and therefore increase their dry-substance content and specific gravity. In other words, they have symptoms that are universally present in any ageing organism.

The process of ageing of *Caenorabditis briggsae* can be delayed if the nutritional substrate is rich in vitamin E or in certain other chemical compounds (8). The latter include certain procaine derivatives, such as Gerovital H_3 (1), which delay formation of the pigment of ageing (lipofuscin). Such information permits one to speculate that a similar phenomenon may also influence the oxidation of lipids in the membranes of all functioning cells. In that case many of the parameters of ageing (specific gravity, water content, turgor, etc.) may be explained by loss of the cell's capacity for controlling water metabolism, as a result of lipid oxidation in cell membranes.

Within that background of information, experiments were performed to see whether symptoms of ageing in plants are measurably related to infection by root-knot nematodes.

MATERIALS AND METHODS

Ultra-low spontaneous luminescence had been shown by Tarusov *et al.* (6) to be an indicator of lipid oxidation. The method they suggested was used in these experiments. Homogenised leaf material was placed in a cuvette with double walls between which water was circulated. The front wall was of transparent quartz of 1 sq cm. Temperature was maintained at 15 ± 1 C.

Luminescence was measured by a

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photomultiplier "FEy-18" with a small photocathode having a spectral sensitivity in the range of 300-600 nm.

For increasing the signal-to-noise ratio, a quartz lens was placed between the cuvette and photocathode to focus the surface of the cuvette onto the photocathode. The window of the photomultiplier was brought to the surface through an opening in a Dewar vessel to prevent absorption of the light rays by the liquid nitrogen. Glass fogging was avoided by working in a room where the temperature could be lowered to about 15 C. Sensitivity, within the range of 400-500 nm was 10-100 quant/sec.

The ultra-low luminescence of tomato, cucumber, and radish plants was studied during ageing and in different stages of infection by *Meloidogyne incognita* (Kofoid and White) Chitwood. The plants, in a standard horticultural soil in pots randomized on a uniformly lighted greenhouse bench, were inoculated with 50 larvae per plant on the 10th day after sprouting. Measurements of luminescence were begun the day after infection and made at intervals thereafter (Table 1).

To assess the effect of infection by root-knot nematodes on the ageing process of plants and to see whether the process is related to lipid oxidation, Xylocaine (Lidocaine hydrochloride, Astra Chemicals Ltd., Mississauga, Ontario, Canada) was used as a lipid antioxidant. In one experiment, tomato plants, seeded and grown as mentioned above, were inoculated at 3 weeks old with 50 larvae per seedling of either *M. incognita* or *M. hapla* Chitwood and one week later were sprayed to run-off with a 0.02% Xylocaine solution, which was repeated at 5-day intervals until four sprays had been applied.

A similar experiment was conducted with radish seedlings infected with *M. incognita*.

RESULTS

The spontaneous ultra-low luminescence in uninfected tomato and cucumber plants increased very little until near the end of the flowering period or the beginning of fruit formation. For radish, that increase coincided with initiation of the flowering shoot. Infected plants, in contrast, had a much earlier increase in luminescence (Table 1). The amount of increase may depend on the degree of nematode infection, for a further increase in the luminescence of some plants was evidenced at about the time that the second generation of nematodes was infecting the plants.

There was no significant difference in leaf mass between infected and uninfected or between treated and untreated tomato, cucumber, and radish plants. There was,

TABLE 1. Effect of *Meloidogyne incognita* infection on luminescence of leaf homogenates of tomato, cucumber, and radish.

Days after infection	Luminescence in quants/sec[x]					
	Tomato		Cucumber		Radish	
	Control	Infected	Control	Infected	Control	Infected
1	22	21	18	19	20	22
2	21	28	19	30	21	27
3	22	40a	18	38a	20	39
4	25	42a	19	40a	21	60a
5	23	55b	20	50a	21	65a
10	22	49b	21	54b	21	70b[z]
20	24	48a	20	53b	45[z]	75b
30[y]	23	60b	18	56b	76	74
45	35	63a	26	70b[z]	75	74
60[y]	39	75[z]	86[y]	76		
75	60[y]	82	80	78		
90	65	90	85	82		

[x]Each figure is an average for 10 plants. Averages followed by *a* or *b* are significantly different from the means of their respective controls at the 5% (a) or 2% (b) levels.
[y]Appearance of a new generation of nematodes, under all plants.
[z]End of flowering or, for radish, the beginning of flowering shoot.

however, a distinct difference in the timing of both the appearance of the flowers and the end of the flowering period. In infected but untreated plants the flowering period began 4-5 days earlier and ended 15-20 days earlier than in uninfected plants. Xylocaine treatment did not shift that timing in healthy tomato plants but brought it back to normal in infected plants (Table 2).

Similarly, Xylocaine-treated uninfected radish plants were not different from controls sprayed with water (Table 3), whereas Xylocaine-treated infected plants differed from water-sprayed plants both in the time of flower-shoot appearance and in the weight of storage roots. A single spraying with 0.02% Xylocaine delayed flowering shoots in infected plants to the normal time of uninfected plants.

TABLE 2. Influence of Xylocaine on tomato plants infected with root-knot nematodes.

Nematode	Treatment	Days flowers present		Water content of leaves[x] %[y]
		First	Last	
Controls	Water	33a	124a	74a
	Xylocaine	31a	126a	75a
M. incognita	Water	27b	103b	69b
	Xylocaine	31a	122a	72a
M. hapla	Water	26b	109b	66b
	Xylocaine	30a	119a	70a

[x]One hour after watering.
[y]Average of 10 plants.
Numbers followed by the same letter within each column of data are not significantly different (P = 0.05).

TABLE 3. Influence of Xylocaine on radish plants infected with *M. incognita*.

	Treatment	Appearance of flower shoots (days)	Weight of bulbs (g)[x]
Controls (no nematodes)	Water	35 (33-36)a	10 (5-15)a
	Xylocaine	35 (34-36)a	11 (6-15)a
M. incognita	Water	22 (20-25)b	1 (0-3) b
	Xylocaine	33 (22-35)a	6 (0-10)c

[x]Average of ten plants, 35 days after seeding.
Means followed by the same letter within each column of data are not significantly different (P = 0.02).

DISCUSSION

This work shows once again that root-knot nematodes provoke profound physiologic changes not only in the affected organ but in the whole plant.

The heightened lipid oxidation in the cell membranes is apparently the result of a disturbance in the balance of the oxidation-reduction processes. Application of an anti-oxidant, such as Xylocaine, tends to normalize those processes in root-knot-infected plants but does not affect them in uninfected plants.

It is noteworthy that a 0.2% solution of another anti-oxidant, propyl gallate ether acid, has been shown to reduce root galling and increase yields of cucumber plants (3).

Those results, with ours, seem to indicate that during root-knot development, just as during ageing, a pathogenic oxidation takes place which can be lessened by applying anti-oxidants.

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