Meloidogyne incognita-Induced Changes in Cell Permeability of Galled Roots[1]

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Abstract: Electrolyte leakage of *Meloidogyne incognita*-infected and healthy tomato roots was compared by conductivity measurements, and by compartmental analysis using ^{86}Rb. Conductivity measurements suggested difference in electrolyte loss from healthy and galled roots. On a percentage basis, a greater rate of efflux occurred for healthy plants, but galled roots contain more electrolytes and may show a larger net loss. Compartmental analysis indicated that: (i) the longer half-time for ^{86}Rb loss from vacuoles of galled root cells could indicate either a greater vacuolar content or decreased tonoplast permeability, (ii) the shorter half-time for loss ^{86}Rb from the cytoplasm of galled root cells could reflect either a reduced cytoplasmic content or an increased plasma membrane permeability, and (iii) in split-root plants, the permeability of the tonoplast and the plasma membrane of cells in nongalled roots is increased by nematode infection on the other half of the root system. Thus, a mechanism for mobilizing minerals to the infection site is proposed. *Key Words*: root-knot nematode, membrane permeability, tonoplast, plasma membrane, electrolyte leakage.

A decrease in amino acids and organic acids, and an increase in sugars in galled-root exudate reported by Wang and Bergeson (20) suggested that the permeability of root cell membranes may be altered by nematode infection. An alteration in the loss of electrolytes from infected plant tissue has been considered by others to be an indication of a pathogen-induced change in membrane permeability (3, 11, 15, 18, 19, 21). Accordingly, we examined the loss of solutes from healthy and galled root tissue using conductometric and compartmental analysis techniques. The latter technique is useful in assessing the permeability of both the plasma membrane and tonoplast. An estimate of the ion content of cytoplasm and vacuoles can also be made by that method.

MATERIALS AND METHODS

Electrolyte loss:—1) Plant culture.—In experiment I, 10-day-old tomato seedlings *Lycopersicon esculentum* Mill. 'Bonny Best' were grown aseptically on filter paper (20) and inoculated with 200 sterile *Meloidogyne incognita* (Kofoid and White) Chitwood larvae per seedling. One week later the root system was excised, rinsed in sterile distilled water, and bathed for 72 h in 25 ml of sterile distilled water containing 50 μg/ml of streptomycin. The conductance of the bathing solution was measured with a conductivity bridge at six 12-h intervals.

In experiment II, 2-wk-old seedlings grown in the greenhouse were inoculated with 10,000 larvae per seedling. Four weeks later galled and healthy roots were rinsed in tap water for 1 h, and surface sterilized by immersion in 0.35% sodium hypochlorite for 3 min. Roots then were rinsed in sterile distilled water, cut into segments, and soaked in the bathing solution prior to determining the conductance as described. A duplicate set of roots was processed without streptomycin.

In experiment III, root systems and basal portions of stems of 4-wk-old seedlings were split longitudinally and transplanted so that each half of the root system grew in a separate pot. One week later, one pot in each pair received 50,000 larvae. Noninoculated single-root plants served as controls. Four weeks later roots were processed and measured for electrolyte leakage as described.

—2) Conductivity measurement.—Electrolyte loss was determined by measuring the conductance of root-bathing solutions initially and at periodic intervals with a conductivity bridge. Experiments were terminated by homogenizing the roots in their bathing solutions and measuring the total conductance of the homogenate. The conductance value (mho) was corrected for initial conductivity of the bathing solution and expressed as a percentage of the homogenate (Δ %H) according to the equation:

$$\%H = (\text{mhos at } t_h - \text{mhos at } t_0 / \text{mhos H}) \times 100\%,$$

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where H = conductivity of homogenate, t_0 = conductivity at time zero, and t_h = conductivity periodically measured (15).

Compartmental analysis:—1) Plant culture.—Plants were grown hydroponically in water, in sand, and in a split-root arrangement in sand. Those grown in water were started on filter paper and were inoculated with 400 surface-sterilized larvae per plant prior to being transferred to 25% Hoagland's solution. Seedlings were grown under fluorescent light with a 16-h photoperiod, and the nutrient solution was replaced daily. After 4 and 6 weeks, ion efflux from roots was determined. Plants grown in sand in a greenhouse were inoculated when 5 wk old with 10,000 larvae/plant. After 2 and 5 wk ion efflux from roots was determined. Roots in the split-root experiment were evaluated 4 wk after inoculation.

—2) Measurement of ^{86}Rb efflux.—Permeability changes of membranes were determined by measuring the efflux of ^{86}Rb from roots. The procedures were as follows: tomato roots were cut into 2-cm segments and washed with distilled water. Segments from infected and healthy plants were separately incubated for 17 h in 200 ml of aerated 25% Hoagland's solution containing 1.25 μmoles K^+/ml labeled with ^{86}Rb (S.A. of 5,000 cpm/μmole K^+). After absorption, the tissue (0.5 - 1.0 g) was blotted, weighed, and placed into a plastic syringe fitted with a rubber tube and pinch clamp to which 10 ml of nonlabeled 25% Hoagland's solution were added. This solution was drained and replaced periodically (washout period) and its radioactivity determined. Initially, the washout period was 30 sec, and the washout time interval was gradually increased to 2 h. The sum of the radioactivity values of the washout samples plus that remaining in the tissue at the end of the washout period was the activity at t_0 (i.e., at start of efflux). Radioactivity was determined with a liquid scintillation counter. Potassium content was measured by a flame photometer. Measurements of K^+ content were made on duplicate samples of root tissue from galled and healthy plants at the start of ^{86}Rb loading, and at the beginning and end of the washout period. Procedures for estimating the amount of radioactivity in different cell compartments and the calculation of the fluxes were those used by others (5, 12, 17, 18).

RESULTS

Electrolyte loss: In experiment I, electrolyte leakage from galled roots was less than in healthy roots (Fig. 1-A). In this experiment the galled roots had been inoculated for only 1 wk. Estimates of percentage of homogenate (Δ%H) after 72 h of incubation, based on Fig. 1-A, were 59 and 75% for galled and healthy roots, respectively. More pronounced

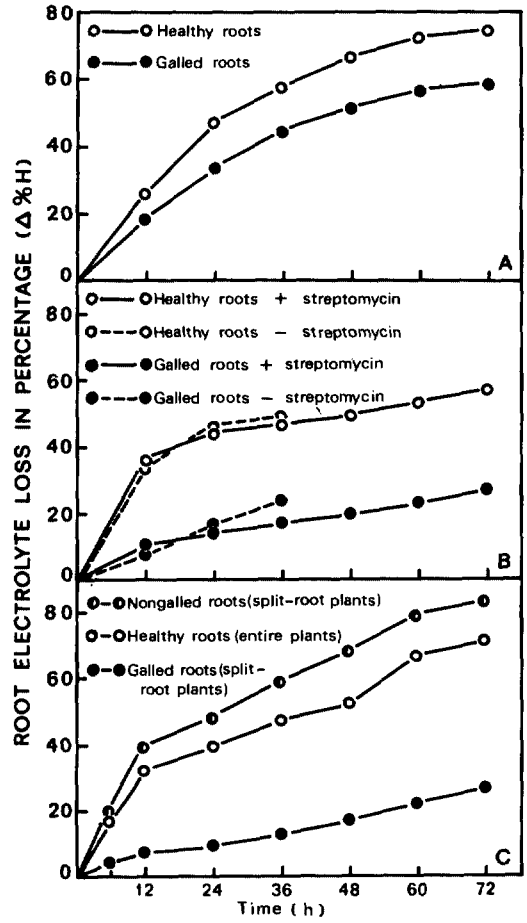

FIG. 1-(A to C) Effects of *Meloidogyne incognita* on electrolyte loss from tomato roots (each point in the graph represents the average of four replications): **A)** changes in conductivity of tomato root-bathing solutions supplemented with streptomycin 1 week after inoculation with 200 sterile *M. incognita* larvae per plant grown in filter-paper culture; **B)** changes in conductivity of tomato root-bathing solutions with or without streptomycin at 4 weeks after inoculation with 10,000 *M. incognita* larvae per plant grown in sand cultures; **C)** changes in conductivity of tomato root-bathing solutions supplemented with streptomycin at 4 weeks after inoculation with 50,000 larvae per plant grown in a split-root culture.

differences in electrolyte loss were obtained in experiment II at 4 wk after inoculation (Fig. 1-B). Streptomycin did not alter electrolyte loss. However, bathing solutions without streptomycin showed a high increase in conductance after incubation for 36 h. This increase appeared to be associated with extensive microbial contamination. Therefore, experiments without streptomycin were terminated after 36 h. The conductance of galled root homogenate was about 60% greater than that of healthy roots in both experiments. This confirms a previous report (13) that galled roots can be a sizeable sink for electrolytes.

By using a split-root technique, both a galled and nongalled root system on a single plant were obtained. Four weeks after inoculation, the electrolyte leakage from galled and nongalled roots of the split-root plants were compared to leakage from single-root noninoculated controls (Fig. 1-C). Nongalled roots from split-root and control plants leaked electrolytes at much greater rate than did galled roots. After 72 h of incubation, electrolyte loss from the nongalled portion of an infected split-root plant was 20% greater than from control roots, whereas loss from the galled portion of split-root plants was 71% less than from controls.

Compartmental analysis: The time course of ^{86}Rb loss, as a percentage of the counts remaining in the tissue, from both galled roots of split-root plants and single-root noninoculated controls at 4 wk after inoculation is shown in Fig. 2-A. Both short-term (dashed lines) and long-term (solid lines) losses indicated that there was a decrease in the rate of ^{86}Rb loss from galled roots of infected split-root plants.

To estimate the loss of ^{86}Rb across the different cell compartment membranes, the data of Fig. 2-A were replotted logarithmically (Fig. 2-B). This plot yielded a straight line at periods from 3 to 12 h, and this slow linear rate of ^{86}Rb loss was interpreted as a first order loss from a specific cell compartment. The compartment contributing to this phase of ^{86}Rb loss is large (containing 65% of the ^{86}Rb in our experiments) and is believed to be from the vacuole (4, 8, 9, 12). Therefore, this portion of the curve characterizes the efflux of ^{86}Rb across the tonoplast membrane. An efflux rate constant for ^{86}Rb loss from vacuoles, K_v, can be calculated from the slope of the linear component of the curve; i.e., K_v = slope × 2.303 and the half-time ($t_{1/2}$) (time required for half of the ^{86}Rb to pass from a cell compartment) for ^{86}Rb loss from this compartment is equal to $0.693/K_v$. The $t_{1/2}$ for ^{86}Rb loss from vacuoles of galled roots of split-root plants and single-root noninoculated controls was 69.0 h and 23.6 h,

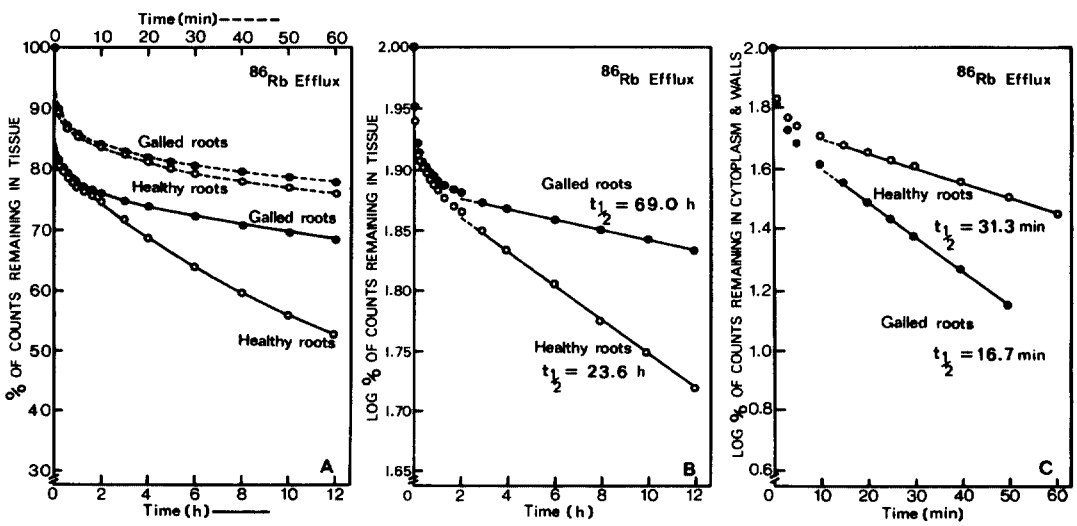

FIG. 2-(A to C). Effects of *Meloidogyne incognita* on ^{86}Rb efflux from both galled roots of split-root plants and single-root noninoculated controls at 4 weeks after inoculation: **A)** dashed lines represent ^{86}Rb losses during the first 60 min, solid lines are for losses from 15 min to 12 h; **B)** logarithmic loss of ^{86}Rb from entire tissue; **C)** logarithmic loss of ^{86}Rb from tissue after subtraction of ^{86}Rb contained in the vacuole.

TABLE 1. Parameters of K^+ fluxes in *Meloidogyne incognita*-infected versus healthy root tissues[a]

Treatment	Time after inoculation (wk)	Root tissue	Half-time[d] ($t_{1/2}$)		K^+ Ion content[e]		K^+ Ion concentration	
			Vacuole (h)	Cytoplasm (min)	Vacuole (μmoles/g)	Cytoplasm (μmoles/g)	Vacuole (mM)	Cytoplasm (mM)
Split-root culture:	4	Healthy[b]	23.6	31.3	32.2	3.6	35.8	72.6
		Galled	69.0	16.7	118.4	0.7	131.5	15.8
		Nongalled[c]	16.4	9.7	22.7	0.9	25.3	18.4
Water culture:	4	Healthy	28.6	25.8	26.8	2.8	29.7	56.2
		Galled	58.7	23.3	50.0	1.4	55.6	28.0
	6	Healthy	55.5	41.4	58.1	8.6	64.6	172.2
		Galled	65.3	34.5	80.2	3.0	89.1	60.0
Sand culture:	2	Healthy	46.6	32.5	37.3	3.0	41.5	61.6
		Galled	68.6	27.0	83.0	1.9	92.3	39.3
	5	Healthy	62.4	52.3	34.5	7.0	38.3	140.9
		Galled	85.4	42.9	85.2	2.1	94.7	42.0

[a]Each value represents the average of two replications.
[b]From single-root noninoculated plants.
[c]Nongalled root system of split-root inoculated plants.
[d]Time required for half of the ^{86}Rb to pass from the tissue under measurement.
[e]Estimates based on 100% equilibration of ^{86}Rb between the cytoplasm and the external solution.

respectively (Fig. 2-B, Table 1).

From Fig. 2-B, extrapolating the linear component of the curve to t = 0 gives an intercept which represents the initial amount of ^{86}Rb in the vacuoles at the beginning of the efflux period. Subtraction of the ^{86}Rb remaining in the vacuole at t = 0 from the total ^{86}Rb in the tissue for various time periods gives the amount of ^{86}Rb in the remaining cell compartments (primarily cytoplasm and cell walls). By replotting the difference logarithmically in the same manner as above, another straight line results after about 15 min. This linear component of the curve is interpreted to represent the loss of ^{86}Rb from the second largest cell compartment–the cytoplasm. The loss is, therefore, characteristic of ion movement across plasma membranes (4, 8, 9, 12). The $t_{1/2}$ for ^{86}Rb loss from cytoplasm was estimated to be 16.7 min for galled roots of split-root plants and 31.3 min for single-root noninoculated controls (Fig. 2-C, Table 1).

Based on the efflux analysis, the K^+ content of vacuoles (Q_v, μmoles/g) and cytoplasm (Q_c, μmoles/g) is calculated by the method of Pierce and Higinbotham (16). The K^+ content of vacuoles (Q_v) and cytoplasm (Q_c) is converted to concentration (mM) by assuming that 1 g of tissue equals 1 ml, and dividing by their respective volume.

The content and concentration of K^+ in cytoplasm and vacuoles, and the $t_{1/2}$ for ^{86}Rb loss from vacuoles and cytoplasm are shown in Table 1. The $t_{1/2}$ for ^{86}Rb loss from vacuoles of galled root cells were from 18-320% longer in all experiments than from healthy roots. Conversely, the $t_{1/2}$ for ^{86}Rb loss from the cytoplasm were from 10-72% shorter than in controls. The content and concentration of K^+ in vacuoles was from 38-421% greater in galled roots and from 20-75% less in the cytoplasm compared to these compartments in control. In nongalled roots of infected split-root plants, the K^+ content of vacuoles and cytoplasm was 30 and 75% lower, respectively, than in vacuoles and cytoplasm of controls.

DISCUSSION

The lower percentage of electrolyte leakage from galled roots was unexpected in view of evidence of increased microbial activity in the rhizosphere of galled roots (1, 7). However, since the concentration of electrolyte in the galled root homogenate was 60% greater than in healthy root homogenate, the absolute amount of electrolyte in the exudate is probably increased, even though on a percentage basis it is less. Thus, an increase in nutrients being released to organisms in the rhizosphere by galled roots appears to be the result of a higher internal concentration of these nutrients, rather than to a significant change in membrane permeability. The abnormally high accumulation of electrolytes

in galled roots was also confirmed by the comparatively higher K^+ level in vacuoles of galled roots. The apparent increased permeability of cells in the nongalled roots of an infected split-root plant may explain the observed disease enhancement of Verticillium and Fusarium wilt (2, 6) when split-root plants were inoculated with the fungus on the nongalled roots. Our results may also explain the mechanism by which minerals such as K^+ are mobilized from nongalled to galled roots (1, 10).

Vacuoles of galled roots contained more ^{86}Rb than those of nongalled roots. Thus, the longer $t_{1/2}$ for ^{86}Rb loss from vacuoles of galled roots could be due to either the greater vacuolar content or a decreased permeability of the tonoplast.

The shorter $t_{1/2}$ for loss of ^{86}Rb from the cytoplasm of galled roots is consistent with the lower cytoplasmic content of K^+. Ingrowths in nematode-induced giant cells (14) may increase the plasma membrane surface area and the rate of ^{86}Rb loss.

The compartmental analysis does involve some assumptions. However, they seem to be reasonable assumptions, and should not invalidate using this method with nematode-infected roots unless the nematodes themselves absorb significant amounts of ^{86}Rb.

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