Total Protein Content of Galled Roots as an Index of Root-Knot Nematode Infestation of Lady's Finger Plants

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ABSTRACT

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Lady's finger plants inoculated with *Meloidogyne incognita* larvae were treated with the water decoction of the leaves of three plant species (*Peristrophe bicalyculata, Tragia involucrata,* and *Anthocephalus kadamba*) and an aqueous suspension of aldrin, a soil insecticide. These four agents had different degrees of nematicidal action as measured by the amount of reduction in root galls and the rhizosphere population of *M. incognita* larvae. The gall index number, which reflected the degree of nematode infection, was positively correlated with percent total protein of galled host plants. The root-gall indices also were positively correlated with the nematode population. The quantity of total galled root protein thus gave a measure of the degree of *M. incognita* infection. The degree of efficacy of a nematicide in reducing root galling also can be measured biochemically through the quantification of total galled root protein.

A comparison between the chemical composition of healthy roots of tomato plants and that of galled roots infected with root-knot nematodes revealed that there was a marked increase in proteins and amino acids in galled roots (2,15). Inside the root galls giant cells or syncytia are formed by the dissolution of cell walls and the coalescing of their contents (7,14). The giant cell cytoplasm contained up to 10 times more protein than normal cell cytoplasm (3).

Elgin et al (8) observed that the level of root galling was directly related to the number of females of *Meloidogyne hapla* within the roots of alfalfa seedlings. The direct relationship between the number of root galls of tomato and lady's finger and population size of *M. incognita* larvae also was reported by Das (6). There is evidence that treatment with nematicidal substances could reduce root galling and suppress nematode populations in the rhizosphere of infected plants (1,16). The objective of the present work was to determine whether protein content of galled roots is a reliable indicator of the degree of nematode infestation.

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MATERIALS AND METHODS

Seeds of lady's finger (*Hibiscus esculentus* (L.) Moench) were surface sterilized and sown, one seed in each of the 72 32-cm-diameter pots containing an autoclaved mixture of clay soil and composted manure (2:1, v/v). Five batches of 12 pots each were infested with *M. incognita* larvae (800/pot) when the seeds were sown, and one batch served as noninfested control. The larvae for inoculation were collected by sieving soil infested with the nematodes. The screenings so obtained were diluted with water, sampled to determine the population density of nematodes, and then applied as inoculum at the rate of 100 ml per pot (16).

Three batches of infested pots were treated 10 days after inoculation with aqueous extracts of three plant species (*Peristrophe bicalyculata* Nees., *Tragia involucrata* L., and *Anthocephalus kadamba* Miq.) and one with aldrin. The authors observed that these three plant species had different degrees of nematicidal activity (*unpublished*). Water decoction of each of the three plant species was prepared by boiling 1 kg of fresh leaves in 2.5 L of water for 20 min (16) and was applied at the rate of 200 ml per pot. Aldrin was applied at 1% aqueous suspension (1 g

commercial aldrin [5% WP] in 5 ml water) at the same application rate. The remaining inoculated batch served as an inoculated, but untreated, control.

Sixty days after planting, all plants were uprooted. The number of root galls on each inoculated plant was counted. After harvest the nematode population in each pot of the five batches of pots was estimated separately (4).

The roots of the test plants were chopped. Three samples of root materials from each batch of test plants were taken at random and the total protein fraction in each sample was estimated separately by Folin's phenol method (5). The root pieces were homogenized and then digested by sodium deoxycholate. Folin's reagent was then added to develop color, the intensity of which was measured by using a colorimeter (Spectronic 20, Bausch and Lomb, Rochester, NY 14625) at a wavelength of 660 nm. The readings were compared against a standard curve of a known protein, bovine serum albumin (Sigma Chemical Company, St. Louis, MO 63178). There were three different dilutions for each of the three samples of root pieces representing a batch of test pots. The average, expressed in terms of percentages of galled-root protein (([wt of protein]/[fresh wt of root]) × 100) was calculated from a total of nine dilutions from each batch.

Two correlations were made: one between the average number of root galls per plant and the final population of *M. incognita* per pot, and another between the average number of galls per plant of a test batch and the percentage of total galled-root protein for that batch.

RESULTS

The effects of the aqueous extracts of *P. bicalyculata, T. involucrata,* and *A. kadamba,* and an aqueous suspension of aldrin on the root galling of lady's finger plants, their galled-root protein content, and the final nematode population in the nematode-infested pots are presented in Table 1. Treatments produced a significant reduction in the number of root galls and in the final population of nematodes. The inoculated untreated plants had significantly more galled-root protein than did the uninoculated untreated ones.

The correlation coefficient between the final population of *M. incognita* and the gall index (average number of galls per plant) was 0.938 (Fig. 1). There was a positive correlation (0.980) between the gall index and the percentage of galled-root protein (Fig. 2).

DISCUSSION

The number of root galls and the number of nematodes in the rhizosphere of treated plants underwent significant reduction (Table 1). This suggests that the plant extracts and aldrin had nematicidal properties. Maximum control of nematode infestation was achieved with *A. kadamba* extract. Least control was achieved with aldrin. The effects of the nematicidal agents were reflected not only in reduced root galling and nematode populations, but also in the quantity of galled-root protein.

The galled-root protein level was directly proportional to the root-gall number as well as the population size of nematodes around the roots of infected plants (Figs. 1 and 2). This biochemical parameter can, therefore, be used as a useful indicator to measure the level of root-knot nematode infestation of host plants. This new

TABLE 1. Decrease in root protein, root galls, and nematode population in the rhizosphere of lady's finger plants inoculated with *Meloidogyne incognita* following treatment with the aqueous extracts of *Anthocephalus kadamba, Tragia involucrata* or *Peristrophe bicalyculata* or an aqueous suspension of aldrin

	Average per plant[a]		
Treatment	Root protein (%)	Root gall (no.)	Final population[b]
Uninoculated	2.2 v	0	0
Inoculated	5.2 z	163.7 x	649.4 x
Treated			
A. kadamba	3.0 vw	58.8 v	88.3 v
T. involucrata	4.1 xy	77.2 v	156.7 vw
P. bicalyculata	3.4 wx	62.7 v	174.7 w
aldrin (1%)	4.5 yz	113.2 w	224.7 w

[a]Average of 12 replicates.
[b]Initial population of *M. incognita* = 800 larvae per pot. Same letters in columns are not significantly different, $P = 0.05$, by analysis of variance.

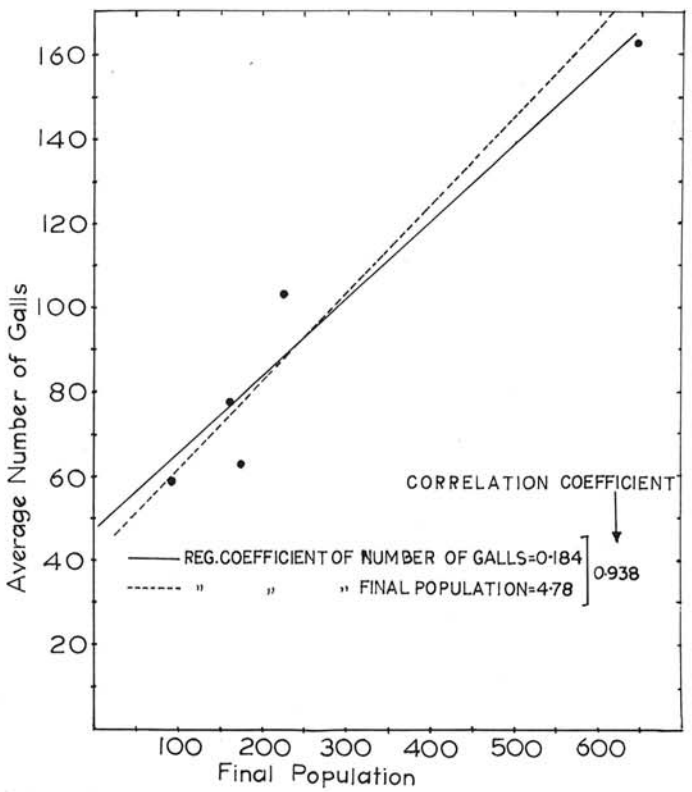

Fig. 1. Correlation between the average number of root galls of lady's finger plants inoculated with *Meloidogyne incognita* (800 larvae per plant) and the final population of nematodes at harvest.

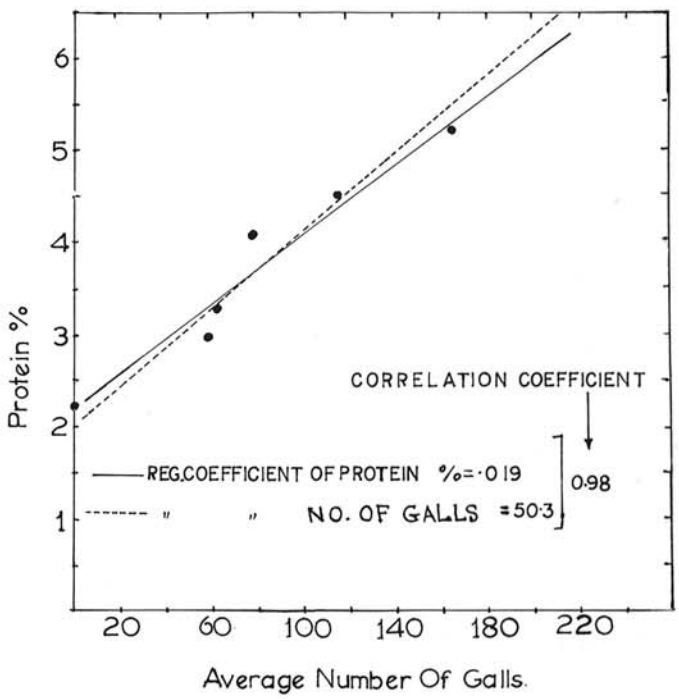

Fig. 2. Correlation between the average number of root galls of lady's finger plants inoculated with *Meloidogyne incognita* (800 larvae per plant) and the percentages of root protein.

indicator could be useful when prolonged and high infestation of nematodes could not be measured because of coalescence of galls. Determination of the exact size of nematode population might be difficult because of soil conditions. By simply measuring the total protein in galled roots, measurements can be made of the degree of *M. incognita* infestation of the host plants and thereby the degree of efficacy of any nematicide.

The levels of plant hormones also are altered by the infection with root-knot nematodes. Matsui and Nakagawa (12) reported that endogenous levels of plant hormones like auxin and cytokinin increased in gall tissues of balsam 1 wk after inoculation with *M. incognita*. Meon (13) observed that cytokinin activity was higher in galled root tissues of tomato plants but lower in xylem exudates as compared with similar measurements in healthy plants.

Auxin in growth-promoting concentration increases RNA synthesis (10). The code of m-RNA is translated into protein following auxin application (11). Thus protein synthesis in galled tissues may be indirectly influenced through changes in the levels of plant hormones by root-knot nematodes. The question remains whether increased protein synthesis in galled tissues results from the direct influence of nematodes or their indirect influence through plant hormones like auxins.

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