Journal of Nematology 19(3):324–330. 1987.

Effect of *Meloidogyne incognita* on Plant Nutrient Concentration and Its Influence on the Physiology of Beans[1]

H. Melakeberhan,[2] J. M. Webster,[2] R. C. Brooke,[2]
J. M. D'Auria,[3] and M. Cackette[3]

Abstract: Phaseolus vulgaris plants, 3, 8, 11, and 13 days old, were inoculated with 0, 2,000, 4,000, or 8,000 second-stage *Meloidogyne incognita* larvae and maintained under controlled conditions. The photosynthetic rate and the shoot and root concentration of K, Ca, Mn, Fe, Cu, and Zn were determined by destructive assay at 1–27-day intervals and by nondestructive assay of leaves, stems, and roots at 27 or 28 days after inoculation. In the destructive assay, the concentration of the elements in the plant tissues did not change until 1 week after inoculation. Thereafter, the trend was mostly decreasing for shoot K and Fe and increasing in the root, whereas Ca had the opposite trend in the shoots. Manganese, Cu, and Fe showed variable trends. Generally, the concentration of K and Mn increased, whereas Ca and Fe decreased, with duration of infection in all treatments. Zinc and Cu decreased in the highest nematode treatments. The overall elemental content generally decreased with level of infection from 1 week after inoculation. Photosynthetic rate based on shoot K concentration significantly decreased with level of infection. In most of the nondestructive assays, the concentrations of shoot K, Zn, and Mn decreased, whereas Ca increased with increasing nematode treatment. One of the first effects of the nematode on host physiology appears to be a change in concentration of nutrient elements in the host plant.

Key words: Phaseolus vulgaris, photosynthesis, root-knot nematode, *Meloidogyne incognita.*

An adequate supply, uptake, and a balanced distribution of nutrient elements within a plant are necessary for normal plant growth. When nematodes infect plants, the nutrient status changes and alters the host physiology. Reports in the literature indicate that the effect of nematodes on the uptake of nutrient elements and distribution within the plant varies with nematode species, host type, and stage of infection (6,8,10,20), whether measurements were taken in the shoots or roots (13,19,26,29) or infected and noninfected parts of a root system (2), and whether data were expressed on a concentration or content basis (15,22,23). Although these reports show that plant-parasitic nematodes change the level and distribution of nutrients within the plant, the experimental data were taken only once during the period of nematode infection and do not establish a relationship with other physiological processes such as photosynthesis.

In a series of studies where *Phaseolus vulgaris* plants of different ages were infected with *Meloidogyne incognita,* reduced rates of photosynthesis and crop yield with increasing levels and duration of nematode infection were found (15–17). These effects were often associated with changes in chlorophyll synthesis and leaf abscission, indicating that the effect of the nematode on the physiological mechanisms associated with photosynthesis may be through its influence on nutrient elements. In this paper, we show the influence of different stages of *Meloidogyne incognita* on the nutrient elements in the shoots and roots of bean plants infected at different ages and explore the relationship between these changes and certain physiological processes in the host.

Received for publication 5 May 1986.

[1] The research was supported in part by a Natural Sciences and Engineering Research Council of Canada grant No. A4679.

[2] Department of Biological Sciences, Simon Fraser University, Burnaby, Vancouver, British Columbia, Canada V5A 1S6.

[3] Department of Chemistry, Simon Fraser University, Burnaby, Vancouver, British Columbia, Canada V5A 1S6.

Correspondence should be addressed to the first author at Division of Nematology, University of California, Davis, CA 95616.

We thank Katheryne Murray for assistance with the elemental analyses.

TABLE 1. Regression slopes for the influence of *Meloidogyne incognita* on the concentration of K, Ca, Mn, Fe, Cu, and Zn in shoots and roots of *Phaseolus vulgaris* over a 27-day period following nematode inoculation.

Days after inoculation	K	Ca	Mn	Fe	Cu	Zn
			Shoots			
1	−2.26	0.28	0.22	−2.26	−0.01	−0.004
3	−3.77	−0.38	−0.03	−0.12	0.01	0.01
8	−11.00	0.11	−0.26	−0.12	0.02	0.003
15	−10.96**	0.04	0.72	−0.03	0.0004	−0.003
22	−14.50*	0.13	−2.32	−0.52	−0.06	−0.03*
27	28.64**	0.15	1.55*	0.01	0.01**	0.02*
			Roots			
1	−7.33	0.14	1.11	−2.06	−0.28	−0.03
3	20.06**	0.30	0.15	1.59	−0.04	0.04
8	12.12	−0.07	1.38	0.15	0.01	0.02
15	24.88**	0.02	−1.53	2.91	−0.004	−0.03*
22	4.50	−0.17	−2.09	3.19*	−0.005*	−0.02
27	7.02	−0.27	−5.98	5.07**	0.08	0.03

Data are from the destructive assay of plant tissues. For clarity of presentation, only regression slopes and significance levels, ** (0.01) and * (0.05), are included here.

MATERIALS AND METHODS

The methods used for growing *Phaseolus vulgaris* L. cv. Topnotch Golden Wax for inoculating with *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 larvae and for determining the CO_2 exchange rate, plant weight, and number of nematodes recovered from the roots were described previously (16,17). Experiments were done on *P. vulgaris* plants of different ages in a single generation of *M. incognita*, and results were obtained by using destructive and nondestructive assay methods.

For the destructive assay, 8-day-old plants were inoculated with 0 (control), 2,000, 4,000, or 8,000 freshly hatched second-stage larvae and the photosynthetic rates were determined followed by harvesting the shoots and roots at 1, 3, 8, 15, 22, or 27 days after inoculation. For the nondestructive assay, plants were inoculated with a similar number of larvae at 3 (first-), 8 (second-), 11 (third-), and 13 (fourth-stage) days after germination and the leaves, stems, and roots were harvested separately at 27 or 28 days after inoculation.

The shoot, or leaf and stem, and root samples were dried separately and ground into powder after dry weights were determined (16,17). Pellets ca. 140 mg/cm^2 were prepared from each sample, and each pellet was analyzed for 30 minutes for K, Ca, Mn, Fe, Cu, and Zn using X-ray energy spectroscopy (27). The reliability of using one side of the pellet was tested as well as the accuracy of reproducing the concentration of the elements. The error incurred by turning either side of the pellet to face the analysis beam was ± 4.1%, and the reproducibility of the concentration of the elements was ± 6.4%.

Data were analyzed using linear regression by comparing the change in concentration of the elements at each sampling date with the number of nematodes either recovered from the roots or inoculated into the soil; to determine changes over time in the destructive assay, the same data were analyzed separately by treatment.

RESULTS

The photosynthetic rate and plant dry weight data corresponding to Tables 1–3 and Figure 1 are reported in Melakeberhan et al. (16,17). The data for the destructive assay and second-stage inoculation of the nondestructive assay experiments are

TABLE 2. Regression slopes for the change in shoot and root concentration (ppm) of K, Ca, Mn, Fe, Cu, and Zn in *Phaseolus vulgaris* over a 27-day period after inoculation with four levels of *Meloidogyne incognita* larvae.

Inoculum level	K	Ca	Mn	Fe	Cu	Zn
			Shoots			
0	873**	−21.8**	88	−15.5	1.100	0.6*
2,000	1,193**	−20.4**	60**	0.7	−0.004	0.5*
4,000	1,229**	−14.3*	68**	10.4	0.200	0.4*
8,000	1,399**	−14.4*	97**	11.9	0.300	0.5**
			Roots			
0	1,227**	−6.8	484**	−419**	−0.8	0.4
2,000	903**	−13.9**	435**	−229**	−1.1	0.1
4,000	1,045**	−14.7**	330**	−220**	2.5*	−0.1
8,000	970**	−18.5**	294*	−199**	4.0**	0.9

Data are from the destructive assay of plant tissues. For clarity of presentation, only regression slopes and significance levels, ** (0.01) and * (0.05), are included here.

based on the numbers of nematodes recovered from the roots; this was shown to be proportional to the inoculum level. The rest of the nondestructive assay experiments are based on inoculum levels.

Destructive assay: Except for an increase ($P \leq 0.01$) in the concentration of root K with increasing number of nematodes 3 days after inoculation, there was no significant change in the other elements during the first week of infection (Table 1). The concentration of shoot and root K ($P \leq 0.01$) at 15 days and shoot K ($P \leq 0.05$) at 22 days following nematode inoculation was lower with increasing numbers of nematodes. Root Zn at 15 days and shoot Zn at 22 days after inoculation decreased significantly ($P \leq 0.05$) with increasing numbers of nematodes per plant (Table 1). At 27 days, however, the concentration of shoot

TABLE 3. Regression slopes of the change in concentration of K, Ca, Mn, Fe, Cu, and Zn in leaves, stems, and roots of *Meloidogyne incognita* infected *Phaseolus vulgaris* inoculated at 3, 8, 11, and 13 days after germination and harvested at 27 (3 day-old) and 28 (all the rest) days after inoculation.

Age at inoculation	K	Ca	Mn	Fe	Cu	Zn
			Leaves			
3	−3.80**	0.03	−0.01	0.04	−0.0004	−0.004*
8	−14.26	0.32	0.06	1.61*	−0.003	−0.003
11	−2.72**	0.05**	0.01	0.01	−0.0001	−0.001**
13	−1.06*	0.09*	0.02	0.55	0.01	0.003
			Stems			
3	−2.01**	0.05	0.01	−0.02	−0.0002	−0.004*
8	−6.72	0.34*	0.23**	0.13	−0.002	−0.02**
11	−0.85**	0.03**	−0.01	0.01	−0.00003	−0.001**
13	0.63	0.08**	0.03**	0.01*	0.001**	−0.001
			Roots			
3	−1.85	−0.02*	−0.10	−0.14	−0.01**	−0.003*
8	−9.21	−0.14*	−0.67	0.26	0.04	0.01**
11	−2.13**	−0.002	0.05**	0.30	−0.002	−0.0002
13	1.13*	0.03*	−0.09**	0.76*	0.01	0.005**

Data are from the nondestructive assay of plant tissues. For clarity of presentation, only regression slopes and significance levels, ** (0.01) and * (0.05), are included here.

K, Mn, Cu, and Zn and root Fe increased significantly ($P \leq 0.05$) with the level of nematode infection.

The change in concentration of each nematode treatment over the duration of infection varied with the element and plant tissue (Table 2). In the controls, the concentration of K and Zn in the shoot and K and Mn in the roots increased ($P \leq 0.05$) while shoot Ca and root Fe decreased ($P \leq 0.05$) with duration of infection. In the nematode-infected plants, the shoot and root concentration of K and Mn increased ($P \leq 0.05$), whereas that of Ca decreased ($P \leq 0.05$). Zinc in the shoots of all nematode treatments and Cu in the roots of the two highest nematode treatments significantly ($P \leq 0.05$) increased while root Fe decreased ($P \leq 0.05$) in all nematode treatments (Table 2).

The total content of nutrient elements was based on their total plant weight and generally decreased from 1 week after inoculation with increasing levels of nematode infection. The total content of elements in the plant significantly ($P < 0.05$–0.01) increased with duration of infection and with decreasing numbers of nematodes. Exceptions were shoot and stem-leaf-root Fe and Cu which did not change significantly. The photosynthetic rate of these plants was expressed on the basis of shoot K concentration. Generally, the photosynthetic rate significantly decreased ($P \leq 0.05$) with increasing level of nematode infection from 1 week after inoculation (Fig. 1).

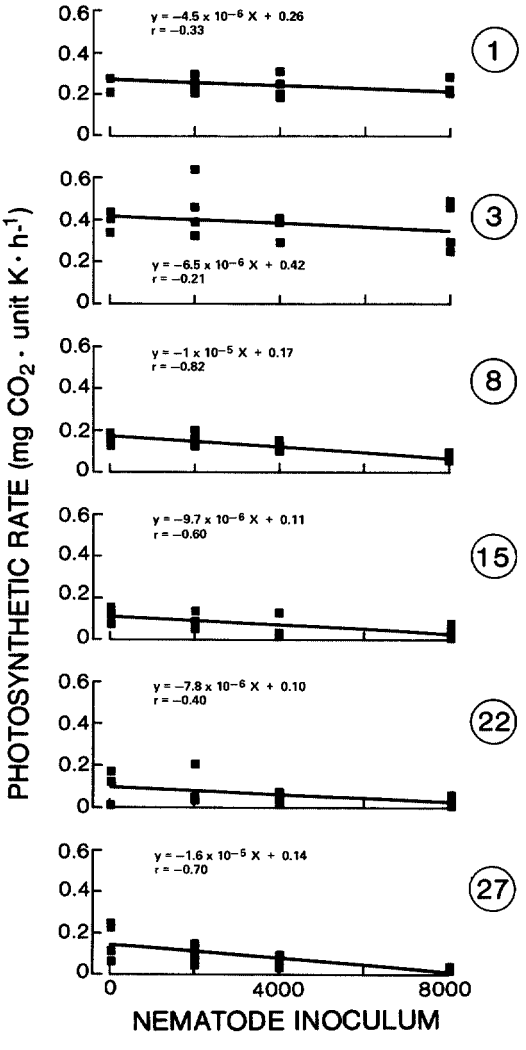

FIG. 1. Influence of *Meloidogyne incognita* on the photosynthetic rate (unit shoot K basis) of *Phaseolus vulgaris* over a 27-day period after nematode inoculation. Data are from the destructive assay. Circled numbers refer to sampling time in number of days after larval inoculation.

Nondestructive assay: In the first inoculation stage (day 3) (Table 3), the concentration of K ($P \leq 0.01$) and Zn ($P \leq 0.05$) in all plant parts and Ca and Cu ($P \leq 0.05$) in the roots was significantly lower with increasing nematode infection. There was no significant change in the concentration of Mn or Fe in any of the plant parts. In the second inoculation stage (day 8) (Table 3), the concentration of Fe in the leaves ($P \leq 0.05$), Mn in the stem ($P \leq 0.01$), and Zn in the roots ($P \leq 0.05$) increased, whereas Zn in the stem ($P \leq 0.01$) and Ca in the root ($P \leq 0.05$) was significantly lower with increasing levels of nematode infection. In the third inoculation stage (day 11) (Table 3), the concentration of K in the leaves ($P \leq 0.01$), stems ($P \leq 0.001$), and roots ($P \leq 0.001$), Zn in the stem ($P \leq 0.01$), and Mn in the roots ($P \leq 0.01$) was significantly lower, whereas stem and leaf Ca ($P \leq 0.01$) increased as the number of nematodes increased. In the last inoculation stage (day 13) (Table 3), the concentration of K in the leaves ($P \leq 0.05$) and Mn in the roots was significantly lower ($P \leq$

0.01); however, Ca in all plant parts, Mn, Fe, and Cu in the stems ($P \leq 0.05$), and Fe and Zn in the roots increased significantly ($P \leq 0.05$) with increasing inoculum level.

The total content of elements in the plant generally decreased with increasing level of nematode infection, and, in most cases, the degree of decline in elemental content was greatest at the first inoculation stage (day 3).

Discussion

This study, monitoring the effect of a single generation of *M. incognita* at intervals on the growth of the plant, showed the changes in both concentration and total content of different elements in bean plants inoculated at different ages to establish a relationship between plant nutrient status and physiological processes such as photosynthetic rate.

The total content of the elements decreased with increasing inoculum level from 1 week after inoculation which corresponded with a decline in plant dry weight (16,17). This is consistent with the results of Price and Sanderson (22) and Price et al. (23), who showed that a lower nutrient content in *Heterodera avenae*-infected barley plants was due to the smaller plant biomass.

In the destructive assay, the nutrient concentration of infected plants generally did not change for more than a week following nematode inoculation, which corresponded with a decline in the photosynthetic rate and chlorophyll content of these plants (16). Over the life cycle of the nematode, however, the trend of this change varied from decreasing amounts of K and Fe in the shoot to increasing amounts in the root and increasing amounts of Ca in the shoot (Table 1). Manganese, Cu, and Zn changed more-or-less equally between shoots and roots. The data show that these changes in host elemental concentration did not affect the nematode population, as was previously observed (11), but did affect host physiology. Melakeberhan et al. (16) showed a decrease in photosynthetic rate on total chlorophyll basis before the chlorophyll content decreased and suggested that some physiological processes might be interfering with chlorophyll function which, in turn, would affect photosynthesis. Furthermore, at later stages of infection, the concentration of nitrogen in these plants increased as the photosynthetic rate and chlorophyll content decreased, which indicated that the nitrogen may occur in a storage form. Considering the role of elements such as Fe and Zn in the synthesis of chlorophyll and Fe, Cu, and Mn in the photosynthetic apparatus, a change in these elements could affect these particular processes, and also influence related physiological processes. The changes in concentration of these nutrient elements in the plant, small as they may be, appear to have a profound effect on the host physiology.

Cations are known to exchange reversibly with identical or different cations dissolved in the soil solution (12), and in this regard the interaction of Ca and K at the absorption, translocation, and (or) metabolic level is a likely example. Potassium, therefore, can be replaced by Ca in such solutions (25,28), and the opposite trends of these two elements suggest that *M. incognita* may be altering the balance of these elements, which may also affect other processes. The lower K concentration in the shoots and leaves of nematode-infected plants, along with increasing numbers of nematodes, usually corresponds with increases in root-galling, giant cell size, and metabolic activity (4,16). This indicates that the uptake and (or) transport of K (2,18,21) and other elements (24) may have been impeded by the deformation of the vascular tissues or *M. incognita* may have utilized K and other elements for its own growth and thereby decreased the supply of elements available to, and concentrated in, the shoot, as shown by the correlation with decreased photosynthetic rate (Fig. 1).

Although the concentration of Ca decreased with duration of infection in the destructive assay (Table 2), it increased with increasing level of nematode infection at the end of most of the nondestructive assay experiments. The increased Ca concentra-

tion in the shoot could be due to two factors. 1) Ca is easily absorbed and readily translocated to the shoot, but once in the shoot, it is less mobile and its concentration therein increases (1). 2) Since these plants were at an advanced stage of chlorosis and leaf abscission, which could result in the disintegration of cellular structures (16), Ca may have been used for linking and modifying cell wall structures (5,14) so that the plant maintains some level of photosynthesis. High concentration of Ca in the shoots of healthy bean plants (3) and other plants (5), or nematode infected potato plants (8), has often been correlated with a high rate of transpiration and suggests direct or indirect damage to the plant by the nematode (7,8). Moreover, high Ca concentration in shoots is known to delay fruit maturity (9), and this could be one of the reasons for the delay in flowering reported in nematode-infected bean plants (17).

In summary, the data indicate that a change in concentration of the nutrient elements in the plant is probably one of the first effects of the nematode on host physiology. These changes in nutrient concentration alter host metabolism and contribute directly (lacking or not in the right form) or indirectly (by affecting other processes) to the chlorosis and premature leaf abscission of infected plants. These effects on the host increase with level and duration of infection and, along with changes in other physiological processes such as photosynthesis, appear to be the main cause of a lower yield in nematode-infected plants. We recognize that biological interactions of different elements can influence host growth, and we examined only a few elements. The change in K concentration, however, seems to be important because of its effect on photosynthesis, either by affecting CO_2 uptake or by altering other key physiological processes such as osmotic potential.

Literature Cited

1. Ayres, P. G. 1984. Effects of infection on root growth and function; consequences for plant nutrient and water relations. Pp. 105–117 *in* R. K. S. Wood, ed. Plant diseases: Infection, damage and loss. Oxford: Blackwell Scientific Publications.

2. Bergeson, G. B. 1966. Mobilization of minerals to the infection site of root-knot nematodes. Phytopathology 56:1287–1289.

3. Biddulph, O., R. Corry, and S. Biddulph. 1959. Translocation of calcium in the bean plant. Plant Physiology 34:512–519.

4. Bird, A. F. 1979. Histopathology and physiology of syncitia. Pp. 155–171 *in* Lamberti and C. E. Taylor, eds. Root-knot (*Meloidogyne* species) nematodes: Biology, taxonomy and control. London, New York: Academic Press.

5. Clarkson, D. T. 1984. Calcium transport between tissues and its distribution in the plant. Plant, Cell and Environment 7:449–456.

6. Evans, K. 1982. Water use, calcium uptake and tolerance of cyst-nematode attack in potatoes. Potato Research 25:71–88.

7. Evans, K., and J. Franco. 1979. Tolerance to cyst-nematode attack in commercial potato cultivars and some possible mechanisms for its operation. Nematologica 25:153–162.

8. Evans, K., D. L. Trudgill, and N. J. Brown. 1977. Effects of potato cyst-nematodes on potato plants 5. Root system development in lightly- and heavily-infested susceptible and resistant varieties, and its importance in nutrient and water uptake. Nematologica 23:153–164.

9. Ferguson, I. B. 1984. Calcium in plant senescence and fruit ripening. Plant, Cell and Environment 7:477–489.

10. Hunter, A. H. 1958. Nutrient absorption and translocation of phosphorus as influenced by the root-knot nematode (*Meloidogyne incognita acrita*). Soil Science 85:245–250.

11. Kirkpatrick, J. D., W. F. Mai, K. G. Parker, and E. G. Fisher. 1964. Effect of phosphorus and potassium nutrition of sour cherry on the soil population levels of five plant-parasitic nematodes. Phytopathology 54:706–712.

12. Loughman, B. 1981. Metabolic aspects of the transport of ions by cells and tissues of roots. Plant and Soil 63:47–55.

13. Maung, M. O., and W. R. Jenkins. 1959. Effects of a root-knot nematode *Meloidogyne incognita acrita* and a stubby-root nematode *Trichodorus christiei* on the nutrient status of tomato, *Lycopersicon esculentum*. Plant Disease Reporter 43:791–796.

14. McEuen, A. R., J. W. Hart, and D. D. Sabnis. 1981. Calcium binding protein in sieve tube exudate. Planta 151:531–534.

15. Melakeberhan, H., R. C. Brooke, J. M. Webster, and J. M. D'Auria. 1985. The influence of *Meloidogyne incognita* on growth, nutrient content and physiology of *Phaseolus vulgaris*. Physiological Plant Pathology 26:259–268.

16. Melakeberhan, H., J. M. Webster, and R. C. Brooke. 1985. Response of *Phaseolus vulgaris* to a single generation of *Meloidogyne incognita*. Nematologica 31:190–192.

17. Melakeberhan, H., R. C. Brooke, and J. M. Webster. 1986. Relationship between physiological

response of French beans of different age to *Meloidogyne incognita* and subsequent yield loss. Plant Pathology 35:203–213.

18. Meon, S., J. M. Fisher, and H. R. Wallace. 1978. Changes in free proline following infection of plants with either *Meloidogyne incognita* or *Agrobacterium tumefaciens*. Physiological Plant Pathology 12:251–256.

19. Nasr, T. A., I. K. A. Ibrahim, E. M. El-Azeb, and M. W. A. Hassan. 1980. Effect of root-knot nematodes on the mineral, amino acid and carbohydrate concentrations of almond and peach root-stocks. Nematologica 26:133–138.

20. Oteifa, B. A. 1952. Potassium nutrition of the host in relation to infection by a root-knot nematode, *Meloidogyne incognita*. Proceedings of the Helminthological Society of Washington 19:99–104.

21. Paulson, R. E., and J. M. Webster. 1970. Giant cell formation in tomato roots caused by *Meloidogyne incognita* and *Meloidogyne hapla* (Nematoda) infection: A light and electron microscope study. Canadian Journal of Botany 48:271–276.

22. Price, N. S., and J. Sanderson. 1984. The translocation of calcium from oat roots infected by the cereal cyst nematode *Heterodera avenae* (Woll.). Revue de Nématologie 7:239–243.

23. Price, N. S., D. T. Clarkson, and N. G. M. Hague. 1982. The uptake of potassium and phosphorus in oats infected with the cereal cyst nematode, *Heterodera avenae* Woll. Revue de Nématologie 5:321–325.

24. Rawsthorne, D., and N. G. M. Hague. 1986. The effect of *Heterodera avenae* on the root system of susceptible land resistant oat seedlings. Annals of Applied Biology 108:89–98.

25. Salisbury, F. B., and C. W. Ross. 1978. Plant physiology. Belmont, CA: Wadsworth Publishing Co.

26. Shafiee, M. F., and W. R. Jenkins. 1963. Host-parasite relationships of *Capsicum frutescens* and *Pratylenchus penetrans, Meloidogyne incognita acrita* and *Meloidogyne hapla*. Phytopathology 53:325–329.

27. Stump, I. G., J. Kearney, J. M. D'Auria, and J. D. Popham. 1979. Monitoring trace elements in the mussel, *Mytilus edulis*, using X-ray energy spectroscopy. Marine Pollution Bulletin 10:270–274.

28. Torimitsu, K., M. Hayashi, E. Ohta, and M. Sakata. 1985. Effect of K and H stress and role of Ca in the regulation of intercellular K concentration in mung bean roots. Physiologia Planatarum 63:247–252.

29. Trudgill, D. L., K. Evans, and D. M. Parrott. 1975. Effects of potato cyst-nematodes on potato plants: Effects of haulm size, concentration of nutrients in Haulm tissue and tuber yield of a nematode resistant and a nematode susceptible potato variety. Nematologica 21:183–191.