Effects of Heavy Metals and *Meloidogyne hapla* on Celery Grown on Organic Soil Near a Nickel Refinery

S. BISESSAR, Plant Pathologist, and R. J. RINNE, Plant Ecologist, Phytotoxicology Section, Ontario Ministry of the Environment, Toronto, Canada M5S 1Z8, and J. W. POTTER, Nematologist, Agriculture Canada Research Station, Vineland Station, Ontario L0R 2E0

ABSTRACT

Bisessar, S., Rinne, R. J., and Potter, J. W. 1983. Effects of heavy metals and *Meloidogyne hapla* on celery grown on organic soil near a nickel refinery. Plant Disease 67:11-14.

A field plot experiment to study the interactive effects of heavy metals (primarily nickel) and the root-knot nematode (*Meloidogyne hapla*) on celery was conducted on a muck farm adjacent to a nickel refinery in southern Ontario. The treatments used were control soil (no metal or nematodes), nonmetal soil plus nematodes (inoculated), heavy metal soil (nickel at 7,500 ppm, copper at 800 ppm, and cobalt at 100 ppm) without nematodes, and heavy metal soil plus nematodes. The nematode treatment alone resulted in an average celery shoot weight 12% less than the controls (significant at $P = 0.05$), whereas heavy metals alone resulted in shoot weight 79% less than the controls. The combined effect of nematodes and heavy metals was a shoot weight 85% less than the controls. The roots of nematode-inoculated plants grown in heavy metal soil had significantly more nematode galls than did the roots of inoculated plants grown in nonmetal soil, indicating that heavy metals, primarily nickel, predisposed celery to greater attack by *M. hapla*.

Additional key words: *Apium graveolens*, crop losses, metal pollution, soil contamination

In a recent paper, soil contamination and toxic effects of the metals nickel (Ni), copper (Cu), and cobalt (Co) to vegetation in the vicinity of a nickel refinery in southern Ontario were described (16). The refinery has been in operation since 1922. Concentrations of Ni up to 15,000 ppm, Cu up to 2,000 ppm, and Co up to 66 ppm occurred in muck soil near the refinery (6). Metal toxicity symptoms (chlorosis, necrosis, and stunting) on crops grown in this contaminated soil have been observed by Phytotoxicology Section personnel and other investigators (6) in the field and in the greenhouse (16). The symptoms have been ascribed mainly to Ni toxicity because of the high levels of Ni in the soil. Ni is regarded as being considerably more toxic to vegetation than either Cu or Co (1,10). Nickel toxicity is a recognized problem in agricultural areas with high soil Ni levels (6, 9, 11).

The northern root-knot nematode (*Meloidogyne hapla* Chitwood) is endemic in polluted muck soils near the nickel refinery. Vegetable crops vary in their susceptibility to infestation by this nematode (14,16). Several reports of effects of pollutants on plant pathogens have been published (8), but relatively few have concerned nematodes. Areas of forest severely damaged by sulfur dioxide (SO_2) and alkaline particulates were found to have higher nematode populations than less damaged areas (2). Ozone and SO_2 inhibited reproduction and development of certain nematode species on soybean (20). Studies with cyst nematodes (*Heterodera* spp.) indicated that several inorganic ions, including Ni^{2+}, stimulated hatching of eggs (4). In a greenhouse bioassay (16), root-knot nematode (*M. hapla*) retarded growth of lettuce leaves by 50% in metal-contaminated soil and 30% in control soils. The effects of nematode infection on growth of celery in the greenhouse study were not statistically significant (16).

To obtain a better understanding of possible interactions between heavy metals and *M. hapla* on celery, an experiment was conducted on a muck farm near the nickel refinery.

MATERIALS AND METHODS

This field trial was conducted on a muck farm about 1 km east northeast of the refinery. Muck soil with relatively low metal levels ("nonmetal soil") was transported to the plot site and placed in an excavated area. Chipboard sheets, turned on edge and buried 35 cm, were used to separate the in situ "heavy metal" soil from the introduced nonmetal soil and from the adjacent soil outside the plot. The plot measured 3.6 × 3.6 m, with each soil occupying half the area.

The soils were homogenized and the initial nematode population, metal concentrations, and pH were determined. Nematode extraction was by the Baermann pan method (18) using 50-ml samples of soil obtained by sampling with a 2.5-cm soil sampling tube. Triplicate soil samples for chemical analysis were collected similarly from each soil. Soil samples were air-dried for 48 hr, sieved through an 80-mesh screen, and stored in screw-topped glass jars prior to chemical analysis.

Chemical analyses were performed by the Inorganic Trace Contaminants Section, Ontario Ministry of the Environment. Nickel, Cu, and Co concentrations were determined using conventional atomic absorption spectrophotometry, and concentrations of sulfur (S), a potential contaminant from refinery SO_2 emissions, were determined by X-ray fluorescence. Soil pH (measured range of 5.8 to 6.6) was determined in a suspension of soil in distilled water (1:1, w/v) with a Corning pH meter.

Seeds of celery, *Apium graveolens* L. 'Utah 52-70,' were sown in 10-cm peat pots containing sandy soil, with one plant per pot. *M. hapla* was cultured on celery plants in the greenhouse. Larvae were extracted from celery roots by mistification (7). Approximately 1,000 second-stage larvae suspended in 7.5 ml of water were injected into the soil around the roots of each 8-wk-old celery seedling with a syringe and hypodermic needle. Control plants were treated with 7.5 ml of water in the same manner. On 13 June 1980, 48 hr after inoculation, 18 seedlings were transplanted into each subplot in three rows 75 cm long, with plants spaced 15 cm apart in the row and rows 45 cm apart. The plot was prepared and planted according to standard commercial practices and irrigated when necessary. Weeds were removed manually.

The treatments were: a) control (nonmetal soil), b) nonmetal soil with *M. hapla*, c) heavy metal soil, and d) heavy metal soil with *M. hapla*.

Pinto and Sanilac bean seeds (*Phaseolus vulgaris* L.) were sown in 15-cm peat pots containing a soil mix consisting of sand,

Accepted for publication 5 May 1982.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0191-2917/83/01001104/$03.00/0

clay, and vermiculite (2:1:1). At the three- to five-leaf stage, these plants were exposed at the plot site to serve as biological monitors of ambient ozone. Plants were replaced each month and rated for ozone symptoms. Bags of sphagnum moss were set up at the plot and at remote control locations for trapping and retaining air pollutants (17). The bags were attached to a plastic supporting bracket and mounted at plant height and at 2 m above ground level on a wooden pole in the center of the plot. After 30 days of exposure, the bags were removed and the moss was processed and analyzed for heavy metals in the same manner employed for vegetation. Celery leaves that exhibited injury symptoms were collected and examined histologically for ozone injury and cultured for fungal or bacterial pathogens. Leaves were surface-sterilized in 0.5% sodium hypochlorite for 2 min, then washed in sterile distilled water. Leaf sections were incubated at 27 C in petri plates containing Difco nutrient agar and 2% malt extract.

The plants were harvested at maturity on 14 August 1980. Four plants were chosen at random from each row of the four subplots for yield determination (total of 12 plants per plot). These same plants were later chemically analyzed. Soil samples (depth 0–15 cm) were collected in triplicate from each subplot for chemical analysis.

Individual plants were cut into shoot and root portions; single shoots and roots were washed free of soil, blotted dry, measured, and weighed (fresh). The average fresh shoot and root weights and heights were calculated. Root galls were counted by direct microscopic examination of roots at ×3 magnification, and roots were indexed for degree of galling on a scale of 0–5 in which 0 = no galls, 1 = 1–5 galls, 2 = 6–25 galls, 3 = 26–100 galls, 4 = 100 galls, and 5 = 100 galls with heavy coalescence. Analyses were made of the chemical contents of three separate leaf, stalk, and root samples. In processing, the vegetation samples were washed with distilled water and oven-dried. The dried samples then were ground in a Wiley mill using an 80-mesh screen. The ground samples were stored in screw-topped glass jars prior to chemical analysis.

RESULTS AND DISCUSSION

Chemical contents of the soils. There were significant differences in Ni, Cu, and Co concentrations between the introduced nonmetal and in situ heavy metal soils (Table 1), reflecting historical contamination from the nickel refinery. Sulfur concentrations were also significantly higher in the heavy metal soil. Elevated Cu levels of 150 ppm occur in muck soil from other areas of Ontario, probably because of heavy addition of Cu-containing pesticides (5). However, the levels found in the muck soil near the refinery were several times higher than those attributed to Cu pesticides. No significant differences in heavy metals occurred between the nonmetal and nonmetal with nematode treatments, nor between the heavy metal and heavy metal with nematode treatments (Table 1).

Chemical contents of celery and toxicity symptoms. Nickel, Cu, and S concentrations in the roots, stalks, and leaves of celery from the heavy metal soil were significantly higher than in celery from the nonmetal soil (Table 2), indicating that considerable uptake from contaminated soil had occurred. Nickel contents of stalks and leaves of plants from the heavy metal soils with nematodes were significantly higher than in stalks and leaves of plants from the heavy metal soil without nematodes, indicating that nematode infestation resulted in an enhancement of Ni uptake from the refinery soil. Conversely, uptake of S apparently was inhibited by nematode infection (Table 2). In a greenhouse bioassay study (16), lettuce grown in contaminated refinery soil that had been autoclaved accumulated significantly more metal (Ni, Cu, Co) than lettuce grown in refinery soil containing *M. hapla*. Celery also accumulated more Ni when grown in autoclaved soil, possibly implying that nematode interference with transport mechanisms in the plant resulted in reduced metal uptake (15). Because the results reported herein do not indicate decreased metal uptake due to nematode infection, it may be that the decrease reported earlier resulted from the autoclaving having altered some additional aspect of the soil that influenced metal uptake.

The heavy metal concentrations in celery generally followed a pattern of root > leaf > stalk (Table 2), as was also observed by other researchers (1,3). Nickel was quantitatively the greatest pollutant among the elements, and the concentrations were believed to be sufficiently high to cause stunting and foliar necrosis. The appearance of the individual treatments in the plot at time of harvest is shown in Figure 1. Injury symptoms, which were observed on both heavy metal treatments, consisted of patchy discoloration of young leaves and yellowing and premature senescence of older leaves beginning from the apexes. Cupping of the leaves and intercostal necrosis were observed 6 wk after transplanting (Fig. 2). These symptoms were not observed on celery grown on the nonmetal soil with or without nematodes, indicating that neither nematode infection itself nor current atmospheric deposition was responsible for the injury. Moss bag analysis showed that amounts of Ni contributed by current deposition were insignificant in relation to historical soil contamination.

Several isolation attempts from the necrotic tissue in conjunction with

Table 1. Chemical contents (ppm, dry weight) of soils at harvest[z]

Treatment	Nickel	Copper	Cobalt	Sulfur
Nonmetal soil (control)	64.0 a	30.7 a	8.3 a	2,200 a
Nonmetal soil + nematode	56.0 a	28.0 a	7.3 a	2,000 a
Heavy metal soil	7,266.7 b	823.3 b	109.7 b	3,400 b
Heavy metal soil + nematode	7,460.0 b	831.0 b	110.3 b	3,600 b

[z] Values are means of three replicates. Within a column, values followed by the same letter were not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 2. Chemical contents (ppm, dry weight) of celery parts at harvest[z]

	Root				Stalk				Leaf			
Treatment	Ni	Cu	Co	S	Ni	Cu	Co	S	Ni	Cu	Co	S
Nonmetal soil (control)	5.2 a	11.3 a	2.7 a	0.14 a	4.3 a	3.3 a	2.2 a	0.17 a	11.5 a	6.0 a	3.0 a	0.71 a
Nonmetal soil + nematode	6.2 a	1.7 b	3.3 a	0.14 a	4.5 a	2.3 b	2.0 a	0.19 a	12.7 a	5.5 a	3.5 a	0.87 a
Heavy metal soil	191.8 b	47.5 c	6.2 b	0.18 b	24.5 b	7.0 c	2.3 a	0.43 b	70.5 b	10.5 b	3.5 a	1.52 c
Heavy metal + nematode	200.2 b	59.7 c	7.0 b	0.20 b	94.8 c	8.5 c	2.2 a	0.33 b	86.3 c	9.2 b	3.2 a	1.28 d

[z] Values are means of 12 replicates. Within a column, values followed by the same letter were not significantly different at $P = 0.05$ according to Duncan's multiple range test.

microscopic examination failed to yield evidence of the involvement of a microbial pathogen. Despite the slight level of bronzing and necrotic stippling observed on the middle leaves of ozone-sensitive Pinto and Sanilac white bean exposed near the plot, histologic examination failed to indicate that ozone-type injury was involved in the chlorosis or necrotic bleaching of the celery leaves.

Although the S content of celery grown on the heavy metal soil was significantly higher than that of the nonmetal soil plants (Table 2), the level was not great enough to have caused injury to celery and was within the range of background S levels in plant foliage in Ontario (13). There were no injury symptoms indicative of SO_2 on sensitive plant species in the vicinity. Sphagnum moss bag chemical analysis showed that aerial deposition contributed very little to the elevated foliar S content, indicating that uptake of S from the soil had occurred.

The pronounced stunting of celery grown on the heavy metal soil and the intercostal chlorosis and necrosis were thus attributed to Ni toxicity. These symptoms were also observed on celery grown on heavy metal soil in the greenhouse (16) and were similar to Ni toxicity symptoms described for other species (1,12,19).

Effects on yield. Average celery shoot weight, shoot height, and numbers of galls are given in Table 3. Shoot weights were significantly different among all treatments, with those in heavy metal treatments showing the least weights. Shoot heights were significantly different except between infected and uninfected celery from nonmetal soils. Comparison of average shoot weights and heights of celery grown on nonmetal soil (control) vs. nonmetal, nematode-infested soil indicated that shoot weight of nematode-infested plants was 12% less and shoot height 6% less than the controls (Table 3). Shoot weights and heights of celery grown on nonmetal soil (control) vs. heavy metal soil (without nematodes) indicated that a 79 and 35% inhibition of weight and height, respectively, could be attributed to metal (Ni) toxicity. The combined effect of *M. hapla* and heavy metal toxicity (treatment d) was a shoot weight 86% lower and a shoot height 47% lower than the controls. The observed combined effect on shoot weight was, therefore, slightly antagonistic (86% observed vs. 91% expected if effects were additive), whereas the combined effect on shoot height was slightly synergistic (47% observed vs. 41% expected). However, these differences were not large enough to be considered significant. With regard to the combined shoot weight 86% lower than the controls, 75% was ascribed to metal toxicity and the remaining 11% to nematode effects. With regard to the combined shoot height 47% less than the controls, metal toxicity was estimated to have caused 40% of the difference, with the remaining 7% attributed to *M. hapla*. Thus, growth of celery was severely retarded by heavy metals, primarily Ni, and the added burden of *M. hapla* caused further growth inhibition.

There were no significant differences in the mean fresh weights of celery roots among the four treatments (Table 3). However, there was a significant negative correlation ($r = -0.89$) between the fresh weight of roots and the number of nematode galls per plant. Gall counts in celery roots from heavy metal, nematode-infested soil were significantly higher than in roots from nonmetal, nematode-infested soil (Table 3). There were also differences in size and shape of galls.

Fig. 1. Plot at time of harvest, showing relative heights of celery plants in each treatment. **Clockwise from left:** Inoculated celery grown on nonmetal soil; uninoculated celery grown on nonmetal soil; inoculated celery grown in heavy metal soil; uninoculated celery grown on heavy metal soil. Moss bags at plant height and 2 m above are attached to a pole in the center of the plot. Bean ozone indicator plants are visible along lower left and upper right sides of plot.

Table 3. Celery shoot weight and height, root weight, and root knot index at harvest (fresh sample)[x]

Treatment	Shoot weight (g)		Shoot height (cm)		Root weight (g)		Root knot index[y]
Nonmetal soil (control)	1,003 a	(···)[z]	62 a	(···)	60 a	(···)	0.8 a
Nonmetal soil + nematode	883 b	(12)	58 a	(6)	53 a	(12)	3.5 b
Heavy metal soil	207 c	(79)	40 b	(35)	57 a	(5)	0.7 a
Heavy metal soil + nematode	136 d	(86)	33 c	(47)	52 a	(13)	5.3 c

[x] Values are means of 12 replicates. Within a column, values followed by the same letter were not significantly different at $P = 0.05$ according to Duncan's multiple range test.

[y] Based on scale of 0–5, in which 0 = no knots, 1 = 1–5, 2 = 6–25, 3 = 26–100, 4 = 100+, and 5 = 100+ knots and heavy coalescence; root knot numbers transformed ($\sqrt{x+1}$).

[z] Values in parentheses represent percentage less than control.

Fig. 2. Celery leaf from heavy metal soil, showing pronounced cupping and intercostal necrotic lesions attributed to nickel toxicity. Symptoms were evident 6 wk after transplanting.

Galls were large, club shaped, and frequently coalesced on plant roots from metal-contaminated soil, in contrast to the small, round galls on roots from the nonmetal soil. Therefore, it may be concluded that heavy metal contamination of soil resulted in an increase in the incidence and severity of root-knot disease on celery.

Other research needs have arisen as a result of this work. Because of the impact of heavy metal toxicity and root-knot nematode on yield, it is important to assess the effectiveness of nematicides in metal-enriched soils. Efforts should also be directed at establishing the mechanism whereby heavy metals predispose celery to root-knot nematode infection.

ACKNOWLEDGMENTS

We are grateful to staff of the Ontario Ministry of the Environment: S. N. Linzon, Phytotoxicology Section, for advice and encouragement; R. Will, Vegetation and Soils Unit, Laboratory Services Branch, for chemical analyses; and B. L. Chai, Phytotoxicology Section, for statistical analysis of the data. We also thank L. I. Wainman, Agriculture Canada, for technical assistance.

LITERATURE CITED

1. Agarwala, S. C., Bisht, S. S., and Sharma, C. P. 1977. Relative effectiveness of certain heavy metals in producing toxicity and symptoms of iron deficiency in barley. Can. J. Bot. 55:1299-1307.
2. Bassus, W. 1968. On the effects of industrial emissions on the population of nematodes in the soil of pine forests. Pedobiologia 8:289-295. (Translated from German)
3. Cataldo, D. A., Garland, T. R., Wilding, R. E., and Drucker, H. 1978. Nickel in plants. II. Distribution and chemical form in soybean plants. Plant Physiol. 62:566-570.
4. Clarke, A. J., and Shepherd, A. M. 1966. Inorganic ions and the hatching of *Heterodera* spp. Ann. Appl. Biol. 58:497-508.
5. Czuba, M., and Hutchinson, T. C. 1980. Copper and lead levels in crops and soils of the Holland Marsh area—Ontario. J. Environ. Qual. 9:566-575.
6. Frank, R., Ishida, K., and Suda, P. 1976. Metals in agricultural soils of Ontario. Can. J. Soil Sci. 56:181-196.
7. Goodey, J. B. 1957. Laboratory methods for work with plant and soil nematodes. Tech. Bull. 2. Her Majesty's Stationery Office, London. 47 pp.
8. Heagle, A. S. 1973. Interactions between air pollutants and plant parasites. Annu. Rev. Phytopathol. 2:365-388.
9. Hunter, J. G., and Vergnano, O. 1952. Nickel toxicity in plants. Ann. Appl. Biol. 39:279-284.
10. Keeney, D. R., Lee, K. W., and Walsh, L. M. 1975. Guidelines for the application of wastewater sludge to agricultural land in Wisconsin. Wisc. Dep. Nat. Resour. Tech. Bull. 88. 36 pp.
11. Lagerwerff, J. V. 1967. Heavy-metal contamination of soils. In: Agriculture and Quality of Our Environment. N. C. Brady, ed. No. 85:353-364. Am. Assoc. Adv. Sci., Washington, DC.
12. Linzon, S. N. 1971. Effects of air pollutants on vegetation. Pages 131-151 in: Introduction to Scientific Study of Atmospheric Pollution. B. M. McCormac, ed. D. Reidel Publishing Co., Dortrecht, Holland.
13. Linzon, S. N., Temple, P. J., and Pearson, R. G. 1979. Sulfur concentrations in plant foliage and related effects. J. Air Pollut. Control Assoc. 29:520-525.
14. Olthof, T. H. A., and Potter, J. W. 1972. Relationship between population densities of *Meloidogyne hapla* and crop losses in summer-maturing vegetables in Ontario. Phytopathology 62:981-986.
15. Seinhorst, J. W. 1979. Nematodes and growth of plants: Formalization of the nematode-plant system. Pages 231-256 in: Root-knot Nematodes (*Meloidogyne* species). Systematics, Biology, and Control. F. Lamberti and C. E. Taylor, eds. Academic Press, London.
16. Temple, P. J., and Bisessar, S. 1981. Uptake and toxicity of nickel and other metals in crops grown on soil contaminated by a nickel refinery. J. Plant Nutr. 3:473-482.
17. Temple, P. J., McLaughlin, D. L., and Linzon, S. N. 1981. Moss bags as monitors of atmospheric deposition. J. Air Pollut. Control Assoc. 31:668-670.
18. Townshend, J. L. 1963. A modification and evaluation of the apparatus for the Oostenbrink direct cotton wool filter extraction method. Nematologica 9:106-110.
19. Wanselow, A. P. 1966. Nickel. Pages 306-309 in: Diagnostic Criteria for Plants and Soils. H. D. Chapman, ed. University of California Press, Riverside.
20. Weber, D. E., Reinert, R. A., and Barker, K. R. 1979. Ozone and sulfur dioxide effects on reproduction and host-parasite relationships of selected plant-parasitic nematodes. Phytopathology 69:624-628.