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Article abstract

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# The influence of chitin-urea amendments applied to an organic soil on a *Meloidogyne hapla* population and on the growth of greenhouse tomato

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This experiment was conducted under greenhouse conditions to evaluate the efficiency of chitin-urea amendments to an organic soil against a Quebec population of the northern root-knot nematode (*Meloidogyne hapla*) and to assess the pathogenicity of this population on tomato *(Lycopersicon esculentum)*. Chitin-urea amendments at 0.2 and 0.4% (vol:vol) were ineffective in reducing the preplant nematode populations. The final *M. hapla* egg populations were significantly increased in chitin-urea amended soils, and a significant positive dosage effect was recorded. Shoot growth of tomato plants was significantly reduced by *M. hapla* but was increased by chitin-urea. At harvest, fruit weights were neither affected by *M. hapla* nor by chitin-urea amendments. The final *M. hapla* egg population was linked to lower N and P levels, and to higher Ca levels in leaf tissues.

**Bélair, G. et N. Tremblay. 1995. Effet d'un amendement de sol à base de chitine-urée sur une population de *Meloidogyne hapla* et sur la croissance de la tomate de serre dans un sol organique. PHYTOPROTECTION 76 : 75-80.**

Cette expérience a été réalisée en serre dans le but d'évaluer l'efficacité d'un amendement à base de chitine-urée appliqué à un sol organique dans le but de réprimer une population du nématode des nodosités *(Meloidogyne hapla)* provenant du Québec et de déterminer le pouvoir pathogène de ce nématode sur une culture de tomate *(Lycopersicon esculentum)*. Les amendements de chitine-urée, aux doses de 0,2 et 0,4 % (vol:vol), n'ont pas réduit les populations du nématode présentes avant la plantation. Les populations finales d'oeufs de *M. hapla* ont été significativement augmentées dans les sols amendés avec la chitine-urée et un effet significatif positif de la dose a été enregistré. Le feuillage de tomate a été significativement réduit en présence de *M. hapla*, et accru par l'amendement de chitine-urée. À la récolte, le poids des fruits n'a pas été affecté par la présence du *M. hapla* ni par la chitine-urée. Les populations finales d'oeufs de *M. hapla* étaient associées avec des teneurs réduites en N et P, mais accrues en Ca dans les tissus foliaires.

## INTRODUCTION

The northern root-knot nematode (*Meloidogyne hapla* Chitwood) parasitizes and reproduces on vegetable crops including tomato (*Lycopersicon esculentum* L.). Several attempts to relate initial *M. hapla* populations to growth and yield of tomatoes resulted in variable responses under both greenhouse and field conditions. Fawole and Mai (1979) reported that *M. hapla* did not limit tomato growth after 6 wk under greenhouse conditions. Under field conditions, Barker *et al.* (1976) reported that yield reductions due to *M. hapla* ranged 10-50% depending on

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the soil type and the geoclimatological area. Olthof and Potter (1977) reported that a density of 27 950 *M. hapla* larvae $kg^{-1}$ of soil delayed tomato fruit ripening. Low to moderate densities (up to 2000 larvae $kg^{-1}$ soil) increased total yield of fruit while densities above 2000 larvae $kg^{-1}$ reduced total yield. Sayre and Toyama (1964) showed that low and medium population densities of *M. hapla* (220 and 1980 larvae $kg^{-1}$ soil) increased the number and weight of processing tomatoes. Stephan (1983) found that a Canadian *M. hapla* population was less damaging to tomato cv. Rutgers than were American and English populations. So far, the pathogenicity of *M. hapla* populations from Quebec to greenhouse tomato has not been investigated.

Root diseases are a major obstacle in organically-grown or pesticide-free tomatoes in Quebec (Carrier 1992). Severe infestations of *M. hapla* occur and are believed to adversely affect growth and productivity. Because this type of production cannot rely on synthetic pesticides for pest management, growers need alternative methods. Among organic soil amendments reported to suppress plant-parasitic nematodes (Rodriguez-Kabana *et al.* 1987), chitin has been effective repeatedly against root-knot nematodes (Mankau and Das 1969; Mian *et al.* 1982). The nematicidal activity of chitin and chitin-urea amendments is attributed to the release of nematicidal levels of ammoniacal nitrogen, combined with the enzymatic activity of chitinolytic microorganisms or with parasitism on plant-parasitic nematodes (Mian *et al.* 1982; Rodriguez-Kabana *et al.* 1984, 1987).

This experiment was conducted to evaluate the efficiency of chitin-urea soil amendment in an organic soil against *M. hapla* and to assess the pathogenicity of a population of this nematode from Quebec to tomato cv. Caruso under greenhouse conditions.

## MATERIALS AND METHODS

Field soil from an experimental carrot (*Daucus carota* L.) plot in Sainte-Clotilde, Quebec, (lat. 45°25' N long. 73°41' W) infested with *M. hapla* was used as inoculum. The soil was a fibrosol with pH 4.5-5.5, 80% organic matter and a C:N ratio of 18:1. Half of the bulk soil was pasteurized for the controls without nematode, and stored for 2 mo at room temperature before use. The experiment was a factorial arrangement of three levels of chitin-urea amendment (ClandoSan 618, Igene Biotechnology Inc., Columbia, MD) at 0, 0.2, and 0.4% vol:vol, and two nematode densities (0 and 1520 *M. hapla* juveniles per 100 $cm^3$ of soil) in a randomized complete block design with five replicates. The nematode population density was estimated by processing ten 100-$cm^3$ subsamples by the modified Baermann pan method (Townshend 1963). The *M. hapla* population density was estimated at 1520 juveniles per 100 $cm^3$ of soil [an average of $532 \pm 86$ juveniles per 100 $cm^3$ soil with an extraction efficiency of 35% (Bélair, unpublished data)].

The chitin-urea granules were mixed in a concrete mixer with field soil and then transferred to 10-L plastic pails (28 cm x 18 cm x 27 cm). Soil moisture was adjusted to field capacity (3 kPa). The pails were placed in a greenhouse at $25 \pm 2$°C for 28 d to allow for partial decomposition of chitin. A 1-mo-old tomato cv. Caruso plant grown in pasteurized organic soil was transplanted in each pail. Pretransplant soil nematode densities were assessed from a 100-$cm^3$ subsample recovered from each pail and processed as mentioned above. The greenhouse conditions were as follows : temperature, $24 \pm 2$°C, and relative humidity, $55 \pm 10$%. Supplementary lighting at 275 µmol $m^{-2}$ $s^{-1}$ PAR was provided by high pressure sodium lamps with a 16-h photoperiod. Plants were watered daily and fertilized weekly with an all-purpose NPK (20-20-20) fertilizer (Plant Product®). Three weeks after transplanting, plants were topped by removing all tissues beyond the 3rd leaf above the last flower cluster in full bloom. Plants were also trimmed twice as follows : 7 wk after transplanting, the first six bottom leaves were removed (1st trimming); 1 wk later, the next three lower leaves were removed (2nd trimming). After drying, the foliage trimmings were weighed and processed for chemical analysis. N and P analyses were done colorimetrically using the Technicon AutoAnalyser II Industrial Method No. 334-74W/B+ (Elmsford, NY).

Analyses of K, Ca, Mg, Fe, Mn, Cu, Zn and B in tissues were done by ICP-AES on a Jarrel-Ash Model ICAP-9000 (Isaac and Johnson 1976).

Starting 10 wk after transplanting, ripe fruits were harvested every 3-4 d and weighed. At the end of the 6-wk picking period, all remaining fruits were removed and weighed. The experiment ended 114 d after transplanting. Roots were carefully separated from soil by shaking and rinsing under running water, dried between paper towels to remove excess water, and weighed. Shoot dry wt from the two trimmings, fresh root weight, yield (total number of fruit, and total fruit weight), root galling (0-10 scale where 0 = no galls, and 10 = 100% galled), and number of eggs plant$^{-1}$ were determined.

Data were analyzed by SAS GLM procedure (SAS Institute 1988) and treatment means were separated by linear contrasts. Nematode data were transformed by ($\log_{10}$ [x + 1]) to achieve homogeneity of variance.

## RESULTS

At transplanting, numbers of *M. hapla* second-stage juveniles ranged 486 -1828 larvae per 100 cm$^3$ in soil samples and were not affected by chitin-urea amendments (Table 1). At harvest, root-knot nematode galling on tomato roots was severe on all nematode-infested plants, with no significant difference between chitin-urea treatment and untreated control. Total *M. hapla* egg populations were significantly higher on chitin-urea treated plants than on control plants, and were higher on plants treated with the high rate versus the low rate.

Weights of trimmed leaves were significantly reduced by *M. hapla* infection but were increased by the chitin-urea treatment at the 1st trimming (Table 2). At the 2nd trimming, leaf weights were significantly greater in chitin-urea treatment than in the control, with a significant positive rate effect. Root weights of *M. hapla* infested plants were increased by 186% on average as compared to the control. Total number and weight of fruits were not affected by chitin-urea treatment or *M. hapla* infestation.

The final *M. hapla* egg populations were linked to lower N and P, but linked to higher Ca in leaf tissues (Table 3). Chitin-urea increased N but reduced P concentrations in leaf tissues. B concentration in leaf tissues was increased by 10% with *M. hapla* while Mn and Zn concentrations were reduced by 400% and 36%, respectively. Fe and Zn concentrations increased with chitin-urea level.

## DISCUSSION

In organic soil, chitin-urea amendments at 0.2 and 0.4% (vol:vol) rates were ineffective in controlling initial and final *M. hapla* population densities on tomato plants. The reproduction rate of the nematode was actually increased in amended soils. Roots may have benefited from an improvement of physico-chemical properties following the amendment (Shelter and Effmert 1987). Top growth of tomato plants was stimulated in chitin-urea treatments, probably as a result of the nitrogen content of the amendment.

**Table 1. Influence of a chitin-urea soil amendment on population density and root galling of *M. hapla* on greenhouse tomato cv. Caruso**

| Chitin-urea rate (% vol:vol) | Initial population[a] (juveniles 100 cm$^{-3}$ soil) | Root galling (0-10)[b] | Final population (x 10$^6$ eggs plant$^{-1}$) |
|---|---|---|---|
| 0 | 1274 | 8.0 | 5.38 |
| 0.2 | 994 | 7.6 | 6.79 |
| 0.4 | 1183 | 8.6 | 13.40 |
| *Contrasts* | | | |
| Chitin vs. without chitin | NS | NS | * |
| Single vs. double rate | NS | NS | ** |

*, ** = significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.
[a] Population at transplanting time.
[b] Root galling index = 1-10 (0 = no galls, 10 = 100% galled).

**Table 2. Growth and yield of tomato cv. Caruso as affected by chitin-urea soil amendment and *M. hapla* infestations**

| Chitin-urea rate (% vol:vol) | Leaf dry wt[a] (g plant$^{-1}$) 1st | Leaf dry wt[a] (g plant$^{-1}$) 2nd | Root fresh wt (g plant$^{-1}$) | Total fruit wt (kg) | Total no. of fruits |
|---|---|---|---|---|---|
| *Without M. hapla* | | | | | |
| 0 | 4.2 | 69.5 | 78.4 | 1.70 | 13.2 |
| 0.2 | 7.2 | 88.6 | 98.7 | 1.70 | 12.4 |
| 0.4 | 8.3 | 89.4 | 93.6 | 1.85 | 13.4 |
| *With M. hapla* | | | | | |
| 0 | 3.2 | 75.8 | 151.3 | 1.80 | 12.4 |
| 0.2 | 4.2 | 86.8 | 133.8 | 1.74 | 14.6 |
| 0.4 | 4.4 | 104.3 | 216.1 | 1.69 | 15.2 |
| *Contrasts* | | | | | |
| *M. hapla* vs. without *M. hapla* | ** | NS | ** | NS | NS |
| Chitin-urea vs. without chitin-urea | * | ** | NS | NS | NS |
| Single vs. double rate | NS | * | NS | NS | NS |
| Interaction | NS | NS | NS | NS | NS |

*, ** = significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.
[a] Total dry wt of leaf per plant at the first and second trimming.

**Table 3. The effect of chitin-urea soil amendment and *M. hapla* on mineral content of tomato leaves recovered from the first trimming**

| Chitin-urea rate (% vol:vol) | N | P | K | Mg | Ca | B | Fe | Mn | Zn |
|---|---|---|---|---|---|---|---|---|---|
| | (g 100 g$^{-1}$ d.m.) | | | | | (μg g$^{-1}$ d.m.) | | | |
| *Without M. hapla* | | | | | | | | | |
| 0 | 3.94 | 1.29 | 2.89 | 0.53 | 3.49 | 97.0 | 88.6 | 260.4 | 15.2 |
| 0.2 | 4.29 | 1.27 | 2.54 | 0.60 | 3.64 | 87.0 | 99.2 | 225.6 | 18.6 |
| 0.4 | 4.56 | 1.23 | 3.24 | 0.57 | 3.37 | 88.2 | 111.6 | 252.8 | 24.6 |
| *With M. hapla* | | | | | | | | | |
| 0 | 3.80 | 1.17 | 2.98 | 0.52 | 3.77 | 107.4 | 91.2 | 64.6 | 10.6 |
| 0.2 | 4.01 | 1.05 | 2.60 | 0.52 | 4.20 | 109.0 | 92.2 | 59.2 | 12.0 |
| 0.4 | 4.27 | 0.95 | 2.37 | 0.57 | 4.35 | 105.8 | 124.0 | 50.0 | 17.2 |
| *Contrasts* | | | | | | | | | |
| *M. hapla* vs. without *M. hapla* | * | ** | NS | NS | ** | ** | NS | ** | ** |
| Chitin-urea vs. without chitin-urea | ** | * | NS | NS | NS | NS | ** | NS | ** |
| Single vs. double rate | NS | NS | NS | NS | NS | NS | ** | NS | ** |
| Interaction | NS | NS | NS | NS | NS | NS | * | NS | NS |

*, ** = significantly different at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Chitin decomposition releases ammonia, which acts as a nematicide with a plasmolyzing effect on the infective second-stage juveniles (Spiegel *et al.* 1987). Chemical soil properties such as pH influence the effectiveness of chitinous amendments (Spiegel *et al.* 1986). In acidic soil, more $NH_4^+$ ion and less ammonia would be present, so again the plasmolyzing effect of ammonia would be lessened. In organic soils, the carbon content is often relatively high; a C:N ratio of 20 or more could inactivate ammonia as a nematoxic compound by stimulating a rapid shift to nitrate (Rodriguez-Kabana *et al.* 1987). Results from this experiment support these hypotheses. Plant yields were not affected by the nematode, nor by the amendments. It is possible that the chitin amendment and soluble fertilization provided more than enough nutrients for plant growth and fruit production.

Populations of specific chitinolytic microflora are stimulated by chitin

amendment, and parasitize the nematode eggs and egg sacs (Spiegel *et al.* 1987). A relationship has been established between the chitinolytic ability of fungi and their capacity to destroy nematode eggs (Rodriguez-Kabana *et al.* 1984). A component of the middle layer of the egg shell of tylenchoid nematode is chitin, or a closely related material (Culbreath *et al.* 1985). There is also some evidence that chitin may be present in the gelatinous matrix of egg masses of *Meloidogyne* spp. (Spiegel and Cohn 1985). However, the unpasteurized organic soil used for this experiment may have not been colonized by this specific type of microflora, given that the chitin amendment was here ineffective.

Soil temperature affects ammonia release following chitin-urea mineralization (Spiegel *et al.* 1988). At 27°C, ammonium concentration peaked after 15 d and almost vanished after 30 d. Nitrate concentration steadily increased throughout the period. When efficient nitrification is established, ammonium is rapidly transformed into nitrate, provided soil temperatures remain warm (Tremblay and Perron 1991). Hence, ammonia in soil can be nematicidal at relatively high concentrations; such concentrations can only be found for a short period of time in warm soil conditions. Therefore, it is possible that, in our experiment, the nematodes were either not in a sensitive stage or not affected by this contact period when soil ammonia reached nematicidal concentrations.

Based on this experiment, it appears that chitin-urea amendments in organic soil do not have the same effect as in mineral soils and do not provide a reliable alternative for the control of *M. hapla*. The treatments used were within suggested rates, ranging 4.4-8.8 t $ha^{-1}$. At a cost of $1.50-2.11 per kg, a grower would have payed out $6000-18 568 per ha. Even if the treatments had been effective, the costs were still 3-10 times higher than any other currently registered nematicide. The poor performance of the chitin-urea treatment in this experiment raises a concern about the cost and relevance of a widespread use of such non-chemical products for the control of plant-parasitic nematodes.

The *M. hapla* population from Quebec reduced top growth but did not affect yield of tomato cv. Caruso. Based on its effect on N, P and Ca leaf composition, the nematode can be seen as a factor causing early senescence of the plant (Been and Schomaker 1986; Walworth and Sumner 1987). It has been suggested that the production of numerous secondary roots from *M. hapla* galled tomato roots and the absence of moisture or nutritional stresses contributed to the lack of effects on growth and yield of tomato under controlled conditions (Fawole and Mai 1979; Sayre and Toyama 1964). Our results support such hypothesis. Furthermore, Fawole and Mai (1979) have also shown that plant age at the time of transplanting and inoculum level of *M. hapla* are not important limiting factors for tomato growth under greenhouse conditions.

More investigations will be needed in the isolation of microorganisms such as bacteria or fungi, and the assessment of their potential as root pathogen of pesticide-free greenhouse tomatoes in Quebec.

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## REFERENCES

**Barker, K.R., P.B. Shoemaker, and L.A. Nelson. 1976.** Relationships of initial population densities of *Meloidogyne incognita* and *M. hapla* to yield of tomato. J. Nematol. 8 : 232-239.

**Been, T.H., and C.H. Schomaker. 1986.** Quantitative analysis of growth, mineral composition and ion balance of the potato cultivar Irene infested with *Globodera pallida* (Stone). Nematologica 32 : 339-335.

**Carrier, A. 1992.** Tomates de serre : une stratégie d'intervention contre les problèmes des racines. Pages 1-10 *in* Journées Horticoles Provinciales [Texte des conférences - Serriculture maraîchère, micro-informatique, culture maraîchère durable], ministère de l'Agriculture, des Pêcheries, et de l'Alimentation du Québec, Québec.

**Culbreath, A.K., R. Rodriguez-Kabana, and G. Morgan-Jones. 1985.** The use of hemicellulosic waste matter for reduction of the phytotoxic effects of chitin and control of root-knot nematodes. Nematropica 15 : 49-75.

**Fawole, B., and W.G. Mai. 1979.** Influence of plant age, light, intensity, nematode inoculum level, and their interactions on tomato growth and reproduction of *Meloidogyne hapla*. J. Nematol. 11 : 199-201.

**Isaac, R.A., and W.C. Johnson. 1976.** Determination of total nitrogen in plant tissue using a block digestor. J. Assoc. Off. Anal. Chem. 59 : 98-100.

**Mankau, R., and S. Das. 1969.** The influence of chitin amendments on *Meloidogyne incognita*. J. Nematol. 1 : 15-16 (Abstract).

**Mian, I.H., G. Godoy, R.A. Shelby, R. Rodriguez-Kabana, and G. Morgan-Jones. 1982.** Chitin amendments for control of *Meloidogyne arenaria* in infested soil. Nematropica 12 : 71-84.

**Olthof, Th.H.A., and J.W. Potter. 1977.** Effects of population densities of *Meloidogyne hapla* on growth and yield of tomato. J. Nematol. 9 : 296-300.

**Rodriguez-Kabana, R., G. Morgan-Jones, and B. Ownley-Gintis. 1984.** Effects of chitin amendments to soil on *Heterodera glycines*, microbial populations, and colonization of cysts by fungi. Nematropica 14 : 10-25.

**Rodriguez-Kabana, R., G. Morgan-Jones, and I. Chet. 1987.** Biological control of nematodes : Soil amendments and microbial antagonists. Plant Soil 100 : 237-247.

**SAS Institute Inc. 1988.** SAS/STAT user's guide : release 6.03. SAS Institute Inc., Cary, North Carolina. 1028 pp.

**Sayre, R.M., and T.K. Toyama. 1964.** The effect of root-knot nematodes on the yield of processing tomatoes. Can. J. Plant Sci. 44 : 265-267.

**Shelter, H., and B. Effmert. 1987.** [Increase of *Globodera rostochiensis* (Wollw.,1923), pathotype 1, with organic matter content and organic or mineral fertilization of the soil] (in German). Arch. Acker-Pflanzenbau Bodenk-D. 31 : 487-493.

**Spiegel, Y., and E. Cohn. 1985.** Chitin is present in gelatinous matrix of *Meloidogyne*. Rev. Nématol. 8 : 184-185.

**Spiegel, Y., E. Cohn, and I. Chet. 1986.** Use of chitin for controlling plant-parasitic nematodes. I. Direct effects on nematode reproduction and plant performance. Plant Soil 95 : 87-95.

**Spiegel, Y., I. Chet, and E. Cohn. 1987.** Use of chitin for controlling plant-parasitic nematodes. II. Mode of action. Plant Soil 98 : 337-345.

**Spiegel, Y., I. Chet, E. Cohn, S. Galper, and E. Sharon. 1988.** Use of chitin for controlling plant-parasitic nematodes. III. Influence of temperature on nematicidal effect, mineralization and microbial population build-up. Plant Soil 109 : 251-256.

**Stephan, Z.A. 1983.** The effects of different densities of *Meloidogyne ardenensis* and of three populations of *M. hapla* on the growth of tomato at four soil temperatures. Nematol. medit. 11 : 93-100.

**Townshend, J.L. 1963.** A modification and evaluation of the apparatus for the Oostenbrink direct cotton-wool filter extraction method. Nematologica 9 : 106-110.

**Tremblay, N., and Y. Perron. 1991.** Transformation de l'urée dans un substrat tourbeux utilisé pour la production de transplants de céleri. Pages 38-43 *in* Actes Symposium 89 Tourbes et tourbières. Diversification et innovation. Volume II - Nouveaux produits. Can. Soc. for Peat and Peatlands, Quebec, Canada.

**Walworth, J.L., and M.E. Sumner.** 1987. The diagnosis and recommendation integrated system (DRIS). Adv. Soil Sci. 6 : 149-188.