

CHITINOUS MATERIALS FROM BLUE CRAB FOR CONTROL OF ROOT-KNOT NEMATODE. I. EFFECT OF UREA AND ENZYMA-TIC STUDIES

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ABSTRACT

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The efficacy of two chitinous materials (Clandosan® 601 and Clandosan® 719) derived from blue crab (*Callinectes sapidus*) wastes for control of *Meloidogyne arenaria* was studied in a greenhouse experiment. Both materials controlled the nematode on 'Summer Crookneck' squash (*Cucurbita pepo*) and on a subsequent planting of 'Rutgers' tomato (*Lycopersicon esculentum*) when preplant incorporated into soil at rates ≥ 10 g/kg of soil. The efficacy of mixtures of urea with Clandosan 601 was explored in a second greenhouse experiment with soil from the same origin. The number of galls/g of fresh squash root (first crop) or tomato (second crop) decreased sharply in response to increasing rates of urea (0-1.0 g/kg of soil) and Clandosan 601 (0-10 g/kg of soil). Treatments with urea alone at rates > 0.5 g/kg of soil were phototoxic; however, combination treatments of urea + Clandosan 601 were not phytotoxic. Treatments with urea alone or with Clandosan 601 alone resulted in increased fresh shoot weights that were proportional to the rates; the heaviest plants developed in soils treated with the combination urea + Clandosan 601 treatments. Soil chitobiase and urease activities after squash were inversely correlated to the number of galls/g of fresh squash root induced by *M. arenaria*, but soil phosphatase activity was not correlated. Enzymatic activities of soil were lower after tomato than after squash. Soil urease activities after tomato were correlated with numbers of galls/g of fresh root, but chitobiase or phosphatase activities were not correlated.

Key words: biological control, *Callinectes sapidus*, chitin, chitobiase, Clandosan, *Cucurbita pepo*, *Lycopersicon esculentum*, *Meloidogyne arenaria*, organic amendments, phosphatase, root-knot nematodes, soil enzymes, urea, urease.

RESUMEN

Rodríguez-Kábana, R., D. Boube y R. W. Young. 1989. Combate del nematodo agallador con materias quitinosas del cangrejo azul. I. Efecto de la urea y estudios enzimáticos. *Nematropica* 19:53-74.

Se estudió en un experimento de invernadero la efectividad de dos materias quitinosas (Clandosan® 601 y Clandosan® 719), obtenidas de caparazones y otros desechos del cangrejo azul (*Callinectes sapidus*), para combatir *Meloidogyne arenaria*. Ambas materias redujeron la incidencia del nematodo cuando se las incorporaron al suelo a razón de ≥ 10 g/kg suelo tanto en el calabacín 'Summer Crookneck' (*Cucurbita pepo*) como en el tomate 'Rutgers' (*Lycopersicon esculentum*) plantado como segundo cultivo después del calabacín. Se

estudiaron también en un segundo experimento con suelo de la misma proveniencia, las efectividades nematocidas de mezclas de urea con Clandosan 601. El número de agallas/g de raíz de calabacín (1r. cultivo) o de tomate (2do. cultivo) disminuyó agudamente en relación directa al aumento en las dosis tanto de urea (0–1.0 g/kg suelo) como de Clandosan 601 (0–10 g/kg suelo). Los tratamientos con urea sola en dosis > 0.5 g/kg suelo resultaron ser fitotóxicos pero no así los combinados de urea + Clandosan 601. Los tratamientos con urea sola o con Clandosan 601 sólo, produjeron aumentos en el peso húmedo de los tallos proporcionales a las dosis utilizadas de estas materias siendo siempre las plantas más pesadas las correspondientes a suelos tratados con los combinados de urea + Clandosan 601. Se demostró una relación inversa entre los niveles de actividad de la ureasa y de la quitobiasa del suelo y el número de agallas/g de raíz húmeda de calabacín aunque no se registró correlación análoga para con las actividades de la fosfatasa del suelo. Las actividades enzimáticas del suelo después del tomate fueron de menor cuantía que las mismas después del calabacín. Se observó una correlación entre las actividades de la ureasa del suelo después del tomate y el número de agallas/g de raíz húmeda de esa planta, cuya relación no se demostró para con las actividades de la fosfatasa o de la quitobiasa.

Palabras claves: *Callinectes sapidus*, Clandosan, combate biológico, *Cucurbita pepo*, enzimas del suelo, fosfatasa, *Lycopersicon esculentum*, mejoradores orgánicos, *Meloidogyne arenaria*, nematodos agalladores, quitina, quitobiasa, urea, ureasa.

INTRODUCTION

The value of chitin amendments to control plant-parasitic nematodes has been demonstrated repeatedly (5,6,8,14,16,26,30,31). Addition of the polymer to soil results in stimulation of a specialized microflora capable of hydrolyzing chitin through production of chitinase and chitobiase (8,16,22,30,31). The activities of these enzymes result in the liberation of N-acetyl-glucosamine. Presumably, other consequent processes result in deamination of the monosaccharide and release of ammoniacal nitrogen into the soil (8,22). The nematocidal activities of chitin amendments is thought to be based on the release of nematocidal levels of ammoniacal nitrogen combined with enzymatic activities of chitinolytic microorganisms on plant-parasitic nematodes (21,24,25). Ammonia in soil can be nematocidal when present at levels exceeding 125 mg N/kg of soil (21,28). Several species of chitinolytic fungi isolated from chitin-treated soils are well-known pathogens of root-knot nematodes (*Meloidogyne* spp.) and other tylenchoid nematodes (7,8,26). These fungi are capable of destroying nematode eggs apparently through the activities of chitinases and other enzymes produced by them (17,18). It is thought that since the egg shells of tylenchoid nematodes contain chitin these fungi can dissolve portions of the shell, penetrate the eggs, and destroy their contents (1,24).

Practical application of chitin for nematode control in soil has been limited because of the relatively high price of the polymer and the large amounts required to obtain consistent nematocidal activity (> 0.5% (w/w)). There are a number of waste products from the crustacean industry available through the world and the amounts of these materials are

increasing exponentially in response to rapid development of shrimp and crab production in aquaculture "farms" (4). The availability of these wastes has stimulated development of industries interested in their utilization. This paper presents a portion of results of research to explore the use of crustacean waste from the crab industry as soil amendments to control plant-parasitic nematodes, and the effects of these amendments on microbivorous nematodes, soil enzymatic activities, and other variables related to soil fertility.

MATERIALS AND METHODS

General procedures: Two experiments were conducted to determine the nematocidal properties of chitinous materials from crab (*Callinectes sapidus*) shells when used to amend soil alone or in combination with urea. Soil for the experiments was a sandy loam (pH = 6.2, 58% sand, 27% silt, 15% clay, organic matter < 1.0%, cation exchange capacity < meq./100 g of soil) from a peanut field at the Wiregrass Substation, near Headland, Alabama. The field was infested with *M. arenaria* (Neal) Chitwood and *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven and had been in continuous peanut (*Arachis hypogaea* L.) for the preceding 10 years with hairy vetch (*Vicia villosa* Roth) as a winter cover crop. Moist (60% field capacity) soil was sieved (1-mm pores) to remove crop debris and large particles and was then mixed 50:50 by volume with builder's sand (< 0.5-mm diam). The mixture, referred to as soil in this paper, was apportioned in 1-kg amounts and placed in 2-L polyethylene bags. The soil in each bag received the appropriate amendment, and after thorough mixing, the contents was transferred to 1-L cylindrical, 25-cm-diam plastic pots. The pots were placed in a greenhouse (25–30 C) where the soils were maintained moist for 10 days before planting (5 seeds/pot) with 'Summer Crookneck' squash (*Cucurbita pepo* L.). After 6 weeks the plants were separated carefully from the soil, and the number of root galls/g of fresh root induced by *M. arenaria* and weights of fresh shoots and roots were determined. After removal of the squash, each pot was replanted with a 3-week-old 'Rutgers' tomato (*Lycopersicon esculentum* Mill.) seedling. Tomatoes were allowed to grow for 8 weeks when the procedure for data collection described for squash was repeated.

Effects of Clandosan on plant growth and nematode populations: A first experiment was conducted to compare the relative nematocidal activity of two chitinous materials derived from crabshells: Clandosan® 601 and Clandosan® 719. Both materials were provided by IGENE Biotechnology (Columbia, MD, U.S.A.) Clandosan 601 consists of demealed crab shells without any further treatment; Clandosan 719 is a product resulting from treatment of demealed crab shells with 1N HCl followed by

neutralization using a patented process (15). Table 1 presents the manufacturer's data which describes the typical elemental composition of these products. In the experiment each material was added to soil at rates of 0, 1, 2, 5, 10, and 20 g/kg of soil. Each rate was represented by eight experimental units (pots) arranged in a randomized complete block design.

Results from the first experiment indicated that Clandosan 601 and Clandosan 719 were most effective against *M. arenaria* at dosages ≥ 10 g/kg of soil. Since these rates were considered uneconomical, a second greenhouse experiment was conducted to determine whether the nematocidal effects of Clandosan 601, the less expensive material, could be improved by inclusion of urea as an additional amendment. In the experiment, Clandosan 601 was applied to soil at rates of 0, 2.5, 5.0, 7.5, and 10.0 g/kg of soil; urea rates were: 0, 0.25, 0.50, 0.75, and 1.0 g/kg of soil. The treatments consisted of all possible combinations of all rates of both materials. Experimental design and all other details for the experiment were as for the first experiment. In the second experiment, additional data were collected on nematode populations in soil and roots. Root systems of both squash and tomato were incubated in water for 72 hours to assess numbers of *P. brachyurus*, and *M. arenaria* second-stage juveniles (J2) (27). Degree of galling in root systems also was evaluated using a subjective scale of 1–5 where 1 = roots with no damage and 5 = necrotic, severely rotted roots. A 100-cm³ soil sample was taken from each pot after removal of squash or tomato to determine nematode numbers (27).

Determinations of soil enzyme activity, pH, and electrical conductivity: A 100-cm³ sample of soil was removed from each pot, air dried at 27–30 C, and kept at 4 C in the dark until enzymatic activities of chitinase

Table 1. Elemental analysis of two chitinous materials derived from crab (*Callinectes sapidus*) shells.

Element	Percent composition	
	Clandosan® 601	Clandosan® 719
Carbon (Total)	22.86	30.48
Carbon (Organic)	17.37	27.13
Hydrogen	4.04	4.76
Oxygen	31.80	32.32
Nitrogen	3.68	5.36
Calcium	19.33	12.23
Phosphorus	1.37	1.72
Magnesium	0.86	0.06
Sodium	0.96	0.16
Potassium	0.11	< 0.06
Iron	0.02	< 0.06

and phosphate, and urease and soil pH and electrical conductivity were determined.

Chitobiase activity (chitobiase acetylamidodeoxyglucohydrolase, E.C.3.2.1.14) was measured using p-nitrophenyl-2-acetiminido-2-deoxy-b-D-glucopyranoside (NPGLu) (Sigma Chemical Co., St. Louis, MO, U.S.A.) as the substrate. Activities measured with this substrate correspond to those of chitobias and hexochitanases (10,34). The method was adapted from that developed by Lederberg for b-galactosidase (12). In the assay, 2 ml of an aqueous solution containing 1 g/L of NPGLu were added to 2 g of dry soil. The suspension was mixed well and placed in an incubator at 37 C for 4 hours. The reaction was stopped by adding 6 ml of 95% ethyl alcohol. After thorough mixing, 10 ml of the suspension was centrifuged at 2 500 g for 20 minutes and 2 ml of the supernatant were pipetted into a test tube with 8 ml of water followed by 1 ml of 0.2 N NaOH. The absorbance of released p-nitrophenol was determined at 420 nm using a Hitachi-Perkin Elmer, Coleman 139 spectrophotometer. The amount of p-nitrophenol was calculated using the appropriate standard curves. Enzymatic activity was expressed in terms of μg p-nitrophenol produced per hour per g of soil. Each determination included controls with soil and no substrate and sterile autoclaved (no enzymatic activity) soil with substrate. Soil phosphatase activity was determined with a modification of the method of Tabatabai and Bremner (33). Two ml of an aqueous solution containing 1 g/L of sodium p-nitrophenol phosphate (U.S. Chemical Corp., Cleveland, OH, U.S.A.) were added to 2 g of dry soil. The suspension was mixed and placed in an incubator for 2 hours at 37 C. The procedure was otherwise as described for the determination of chitobiase activity. Phosphatase activity was expressed in terms of μg of p-nitrophenol formed per hour per g of soil.

Soil urease activity was assayed as described elsewhere (23) using urea as substrate by determining the amount of amoniacal N released with a microdifusion procedure (3). Urease activity was defined in terms of μg N liberated per hour per g of soil.

Soil pH was determined by mixing 10 g of dry soil with 10 ml of demineralized water. The pH of the suspension was determined after 5 minutes using a Corning Model 12 pH meter. The soil-water suspension received an additional 10 ml of demineralized water and after thorough mixing 10 ml of the diluted suspension was centrifuged (2 500 g for 20 minutes). Conductivity of the supernatant was determined using a Wheatstone bridge equipped with a conductivity cell ($k = 1.0$).

Statistical analysis: All data were analyzed following standard procedures for analysis of variance (32); Fisher's least significant differences were calculated when *F* values were significant and are included in the figures. Curve fitting was by the least square method (11). Unless other-

wise stated, all differences referred to in the text were significant at $P \leq 0.05$.

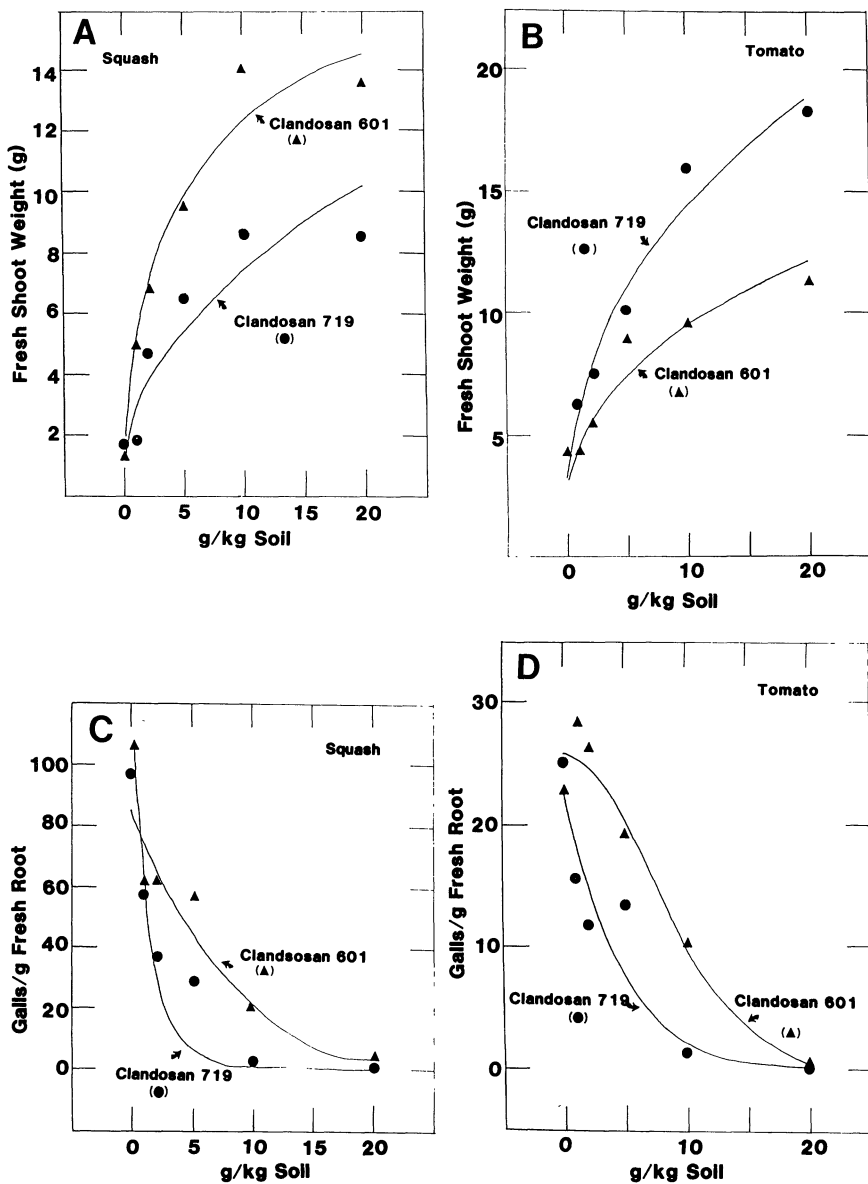


Fig. 1. Relation between rates of two chitinous amendments Clandosan 601 and Clandosan 719 and fresh shoot weight and galling of squash and tomato infected by *Meloidogyne arenaria*. A) Fresh shoot weight of squash. B) Fresh shoot weight of tomato. C) Number of galls produced on squash. D) Number of galls produced on tomato.

RESULTS

Data on plant response and relative nematicidal activity of Clandosan 601 and Clandosan 719 are presented in Figure 1A–D. Application of both materials resulted in an exponential increase in fresh shoot weights of squash (Fig. 1A) and tomato (Fig. 1B) in a manner described best ($R^2 = 0.89$) by:

$$Y_s = Ae^{[(B-\ln X)^2/C]} \quad \text{I}$$

where Y_s represents fresh shoot weights in g, X the dosage of amendment in g/kg soil, and A , B , and C are constants. Squash shoot weights were higher in response to Clandosan 601 than to Clandosan 719; however, shoot weights of tomato were heaviest in soils treated with Clandosan 719.

The number of galls/g of fresh root (Y_g) induced by *M. arenaria* was inversely related to the rates of Clandosan 601 or Clandosan 719. The pattern of response to dosages of the materials was adequately ($R^2 = 0.92$) described by:

$$Y_g = Ae^{[(X-B)^2/C]} \quad \text{II}$$

where A , B , and C are constants.

Gall data indicated that Clandosan 719 was more effective than Clandosan 601. Root systems of either squash (Fig. 1C) or tomato (Fig. 1D) grown in soils treated with the two highest rates of Clandosan 719 had essentially no galls; this was true also for Clandosan 601 but only at the 20-g rate.

Urea-Clandosan 601 Experiment

Squash: The addition of Clandosan 601 to soils resulted in increased fresh weights of shoots (Fig. 2A). Factorial analyses of the data for this variable evidenced a significant Clandosan 601 \times urea interaction. This was reflected in the fact that responses to increasing rates of urea to Clandosan 601 dosages did not follow the same pattern for all urea rates. Whereas fresh shoot weights increased linearly in response to increased rates of Clandosan 601 in the no urea treatment, shoot weights of plants in soils with urea + Clandosan 601 at the 2.5-g rate attained 70–90% of the maximum weight observed in the experiment with little additional increase observed in response to higher rates of Clandosan 601.

All treatments with urea alone but one (0.75-g rate) resulted in root systems with reduced vigor when compared to the control (Fig. 2B); however, in general, the combination with Clandosan 601 improved root condition for all urea rates. The Clandosan 601 \times urea interaction for root condition index was not significant. A general pattern of response in root condition index values (R_i) to Clandosan 601 rates fitted ($R^2 = 0.94$) the hyperbolic model:

$$R_i = 2.622 + 0.015/X \quad \text{III}$$

Applications of urea alone resulted in fewer galls/g of fresh root only

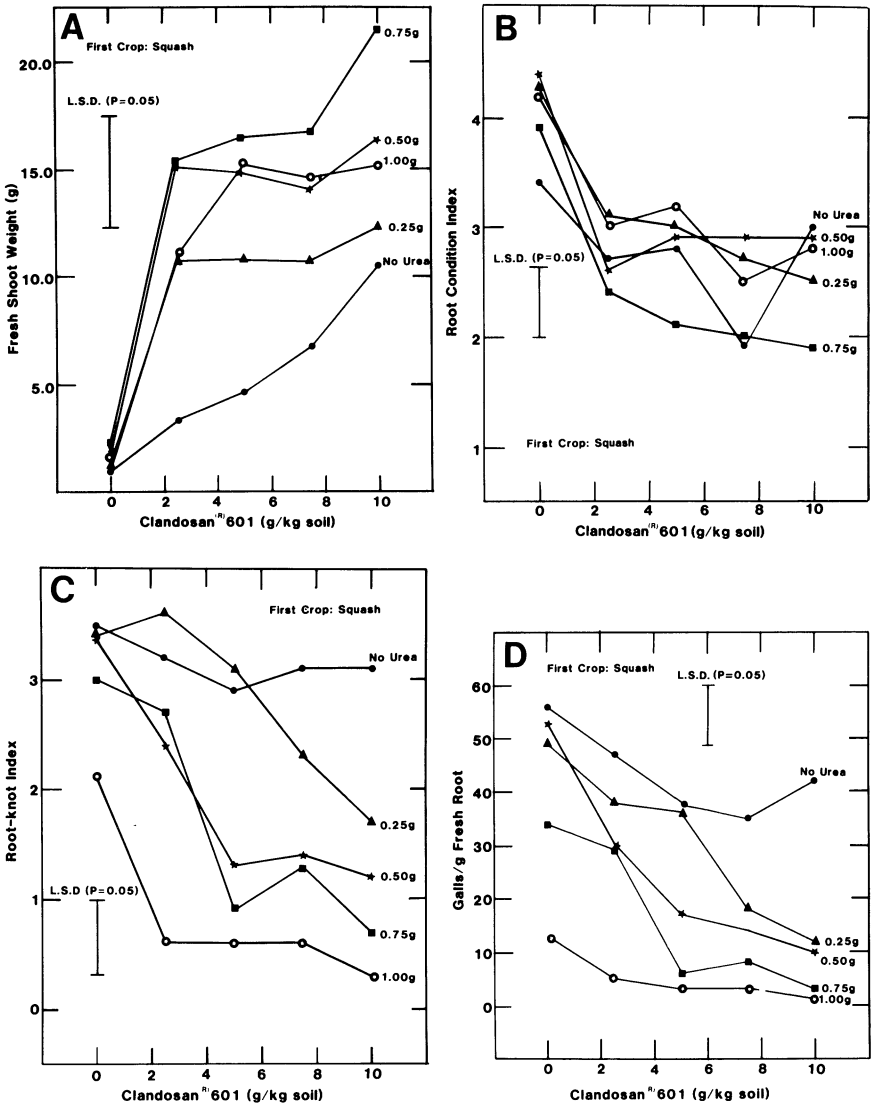


Fig. 2. Effects of Clandosan 601 and urea soil amendments on fresh shoot weight, root condition, and galling of squash infected by *Meloidogyne arenaria*. A) Fresh shoot weight. B) Root condition based on a 1–5 scale where 1 = roots with no damage and 5 = severely deteriorated roots. C) Root-knot index where 0 = 0 galls and 10 = maximal galling. D) Number of galls.

when applied at rates of 0.75 or 1.0 g/kg of soil (Fig. 2D). This pattern of response also was true for root-knot index (Fig. 2C) but only for the 1.0-g rate. The addition of Clandosan 601 to soil with urea resulted in lower numbers of galls and root-knot indices than in roots of plants

from soils with urea alone. Roots from soils treated with Clandosan 601 alone had fewer galls/g of root with increasing rates of the material. A corresponding reduction in root-knot indices also was observed but was significant at $P = 0.10$. The interactions of Clandosan 601 \times urea were significant for both root-knot index and number of galls/g of root.

All treatments with urea alone but one (0.5 g/kg soil) reduced numbers of *Meloidogyne* juveniles/g of fresh root (Fig. 3A). Amendments with Clandosan 601 and no urea decreased root populations of *M. arenaria* (J2) only at the two highest rates; the 2.5- and 5.0-g rates increased numbers of J2 recovered from roots. Roots from soils with the 1-g rate of urea produced no *M. arenaria* J2 regardless of whether urea was used alone or in combination with Clandosan 601. Increasing rates of Clandosan 601 in combination with urea at the 0.5-g or the 0.75-g rates resulted in hyperbolic declines in *M. arenaria* J2 numbers in the roots; this relation was not true for the 0.25-g rate of urea. The Clandosan 601 \times urea interaction for *M. arenaria* J2 populations recovered from roots was significant.

Population levels of *P. brachyurus* in roots were affected adversely by urea and Clandosan 601 (Fig. 3B). The interaction Clandosan 601 \times urea on this variable was not significant. There was a general pattern of decline in numbers of *P. brachyurus* in the roots in response to increasing rates of Clandosan 601 or urea.

Soil populations of either *M. arenaria* J2 or *P. brachyurus* were low ($< 20/100 \text{ cm}^3$ of soil) for all treatments and no pattern of response to rates of either Clandosan 601 or urea could be established (data not presented). Soil populations of microbivorous nematodes (Rhabditida) were unaffected by Clandosan 601 treatments with no urea, but their numbers were reduced in soils with urea and no Clandosan 601 except for the 1.0-g rate of urea (Fig. 3C). Populations of these nematodes in soils treated with the three highest rates of urea increased in response to increasing rates of Clandosan 601. The interaction Clandosan 601 \times urea for microbivorous nematodes in soil was significant.

Soil pH increased in response to increasing rates of Clandosan 601 regardless of urea level (Fig. 4A). In general, the inclusion of urea in the amendments reduced pH values; the degree of reduction observed was dependent on individual urea + Clandosan 601 combination rates so that the interaction for Clandosan 601 \times urea for this variable was significant.

For all levels of urea, electrical conductivity of the soil water extracts increased in response to increasing rates of Clandosan 601 (Fig. 4B). Urea had some effect on conductivity values, but no general pattern of response to the chemical could be established. The Clandosan 601 \times urea interaction was significant.

Soil chitinase activity was not affected by the addition of urea with-

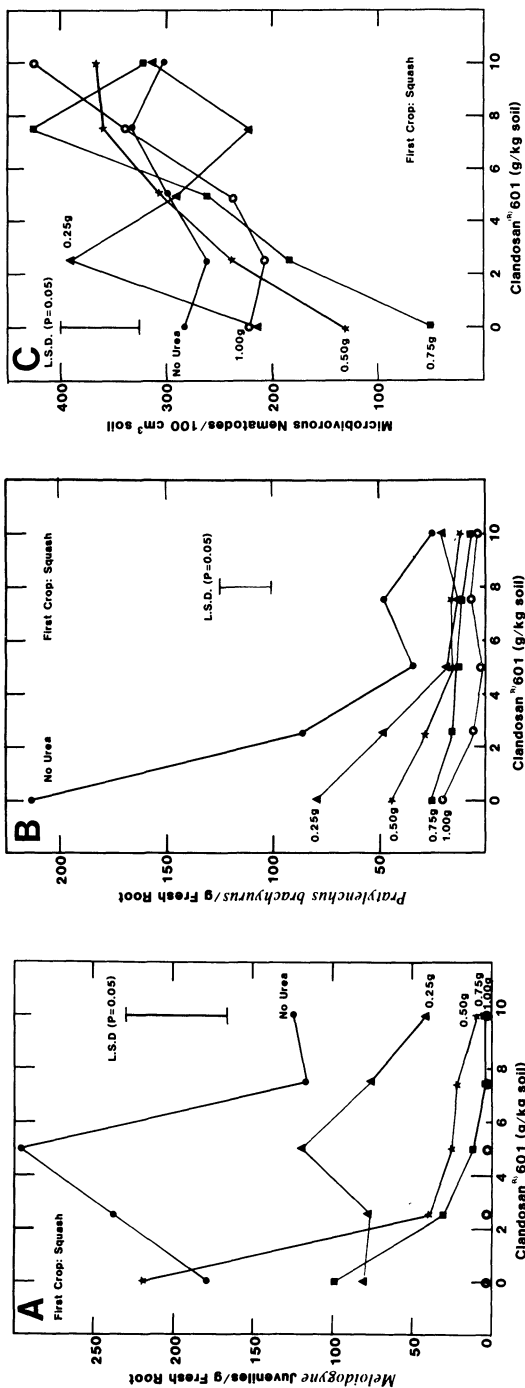


Fig. 3. Effect of Clandosan 601 and urea amendments on nematode populations associated with squash. A) Numbers of *Meloidogyne arenaria* J₂ recovered from roots. B) Numbers of *Pratylenchus brachyurus* recovered from roots. C) Microbivorous nematodes in soil.

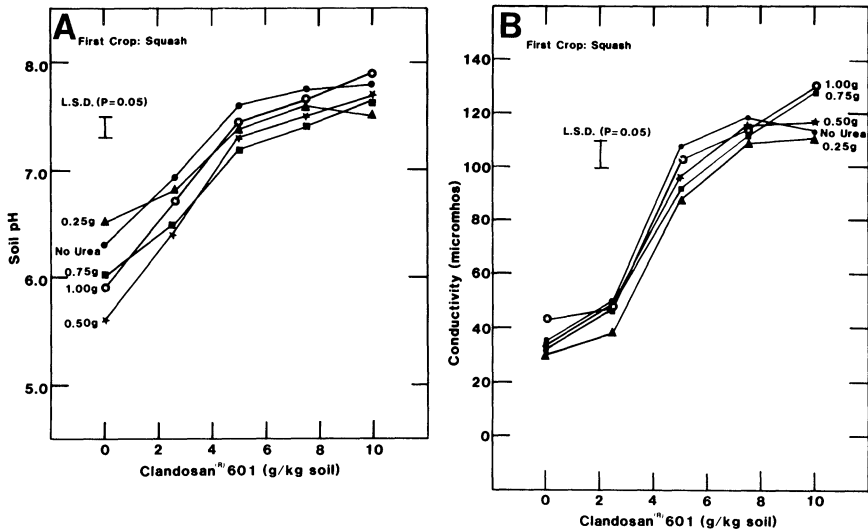


Fig. 4. Relation between soil amendment rates of Clandosan 601 and urea on soil pH (A) and electrical conductivity of soil water extract (B) following growth of squash.

out Clandosan 601 (Fig. 5A); however, in all soils with urea there was a general pattern of increased enzymatic activity in response to increasing rates of Clandosan 601. The Clandosan 601 \times urea interaction on chitobiase activity was not significant. The relation between soil chitobiase activity and Clandosan 601 rates was linear and positive when considered independently of the effect of urea on the variable (Fig. 5B). The effect of urea on this enzyme activity also was positive and linear when considered independently of Clandosan 601 rates (Fig. 5C).

Soil urease activity was stimulated by the addition of urea at rates $>$ 0.50 g/kg of soil but only in soils that received Clandosan 601 at levels $>$ 2.5 g/kg of soil (Fig. 6A). Since there was no Clandosan 601 \times urea interaction on urease activity general patterns of response to urea or Clandosan 601 were established. Urease activity increased in response to increasing dosages of either urea or of Clandosan 601 (Fig. 6B).

Soil phosphatase activity was much more variable than the other two enzymatic activities measured (Fig. 6C). No distinct pattern of response to either urea or Clandosan 601 was detected. The Clandosan 601 \times urea interaction of phosphatase activity was significant.

The number of galls/g of squash root was inversely related to chitobiase and urease activities of soil but not to phosphatase activity (Fig. 7A,7B).

Tomato: The number of galls induced by *M. arenaria* in tomato roots in soils with Clandosan 601 and no urea was not affected by the amount of Clandosan applied (Fig. 8A). The addition of urea to soil without

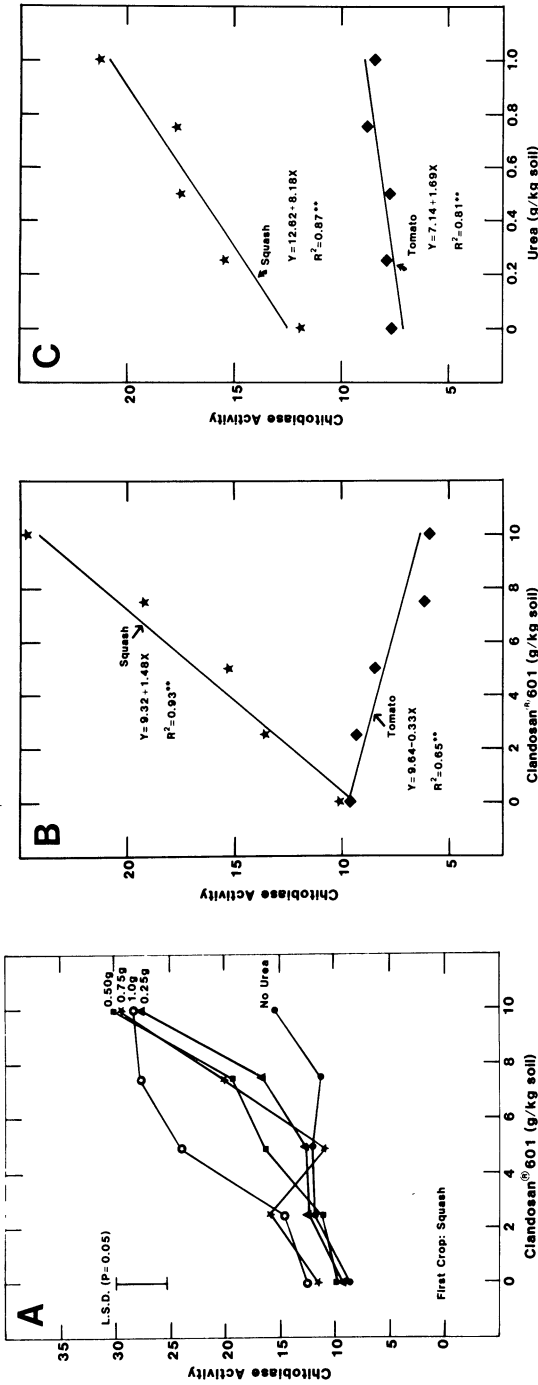


Fig. 5. Effects of amendments with Clandosan 601 and urea on chitinase activity of soil with squash or tomato. A) Combined effects of amendment rates on enzyme activity after squash. B) Effect of Clandosan 601 rates on chitinase activity after squash and after tomato. C) Effect of urea on chitinase activity after squash and after tomato.

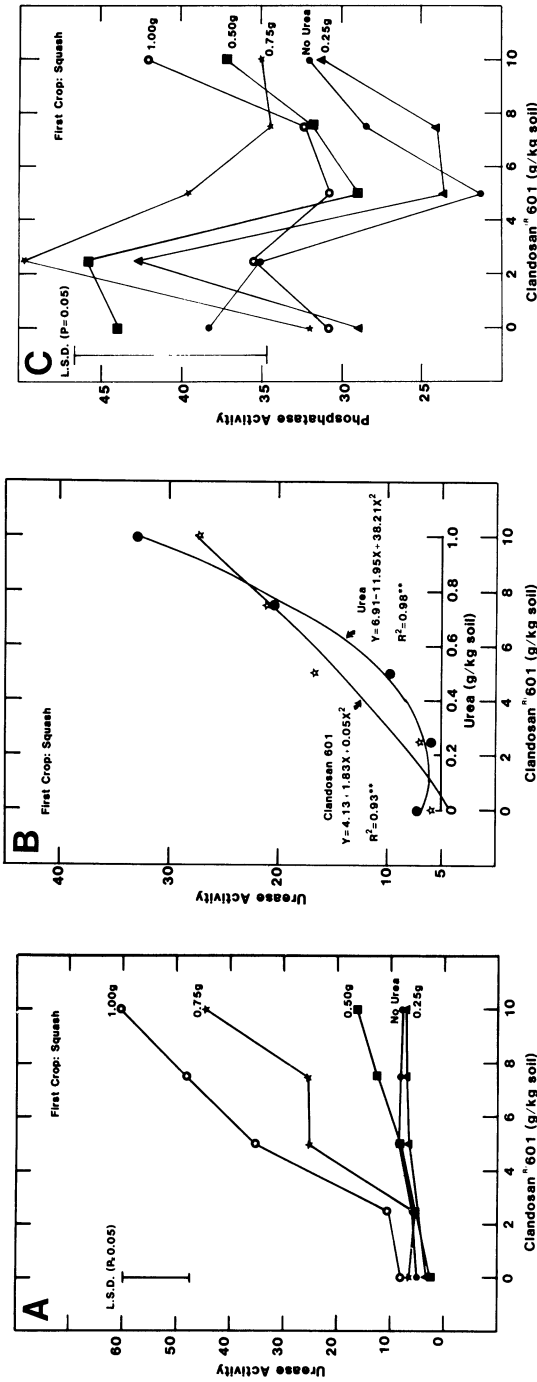


Fig. 6. Effects of amendments with Clandosan 601 and urea on soil urease and acid phosphatase activities following squash. A) Combined effects of amendment rates on urease activity. B) Generalized effect of Clandosan 601 and urea on urease activity. C) Combined effects of amendment rates on acid phosphatase activity.

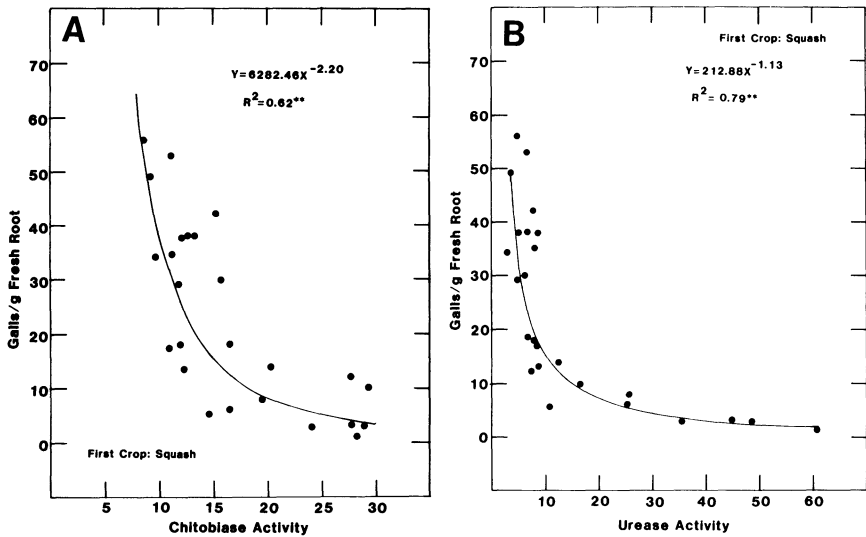


Fig. 7. Relation between number of galls induced by *Meloidogyne arenaria* in squash and enzymatic activities on soil planted to the crop. A) Chitinase activity. B) Urease activity.

Clandosan 601 resulted in lower numbers of root galls when the compound was applied at the 0.75- and 1.0-g rates. All treatments with Clandosan 601 + urea resulted in linear declines in gall numbers in direct response to Clandosan 601 rates > 2.5 g/kg of soil. The Clandosan 601 \times urea interaction for gall numbers was significant. Data for root-knot index values followed the pattern described for galls/g of root and are not presented. Also, there was a general improvement in root condition in response to increasing amounts of Clandosan 601 at all urea rates (data not presented). The numbers of *M. arenaria* J2/g of tomato root was either unchanged or increased in response to applications of Clandosan 601 with no urea (Fig. 8B). When urea was included there was a general decline in numbers of J2/g of root in response to increasing rates of Clandosan 601. The interaction Clandosan 601 \times urea for *M. arenaria* J2 recovered from roots was significant.

Root populations of *P. brachyurus* were affected adversely by increasing amounts of Clandosan 601 in soil with no urea (Fig. 8C). Applications of urea at rates > 0.25 g with no Clandosan 601 also reduced root populations of the nematode. Numbers of *P. brachyurus* in roots from soils with 1.0 g of urea were not reduced further by addition of Clandosan 601; however, in soils treated with the 0.25-g rate *P. brachyurus* populations were reduced proportionately with increasing rates of Clandosan 601.

Soil populations of *M. arenaria* or *P. brachyurus* were low (< 30/100 cm³ of soil) and no pattern of response to either urea or Clandosan 601

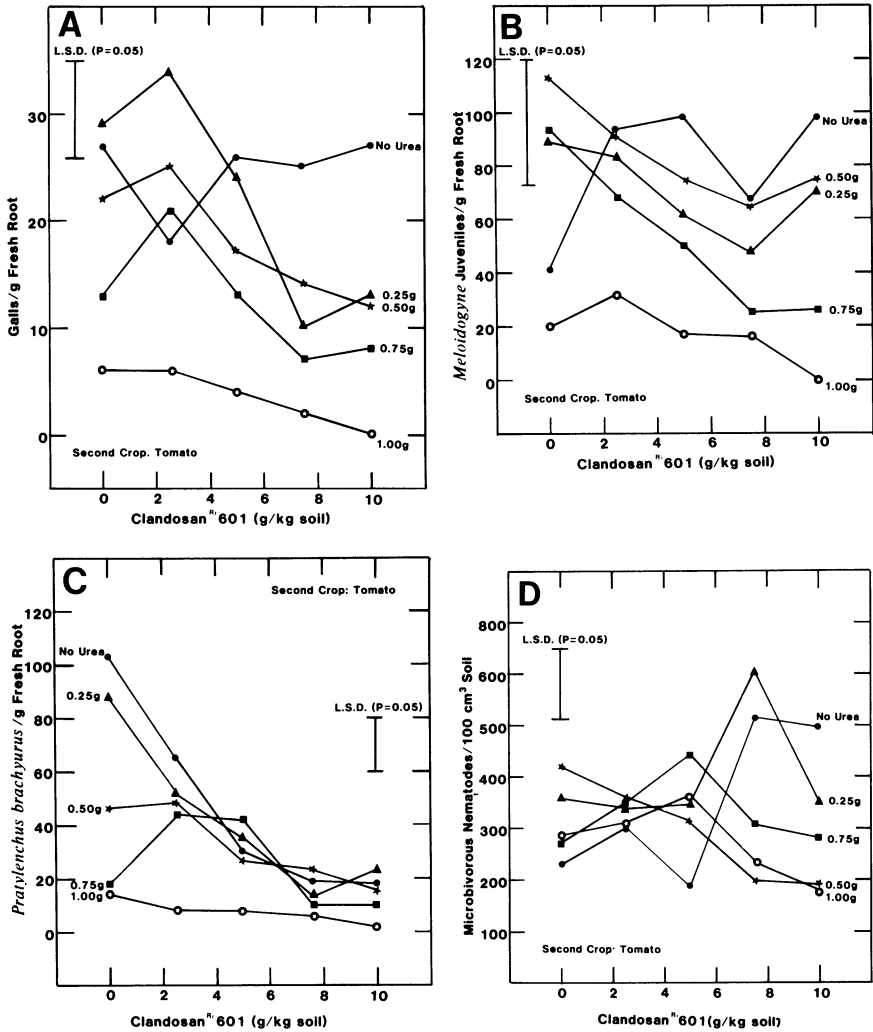


Fig. 8. Effect of Clandozan 601 and urea amendments on galling and nematode populations associated with tomato. A) Number of *Meloidogyne arenaria*-induced galls. B) Number of *M. arenaria* J2 recovered from roots. C) Numbers of *Pratylenchus brachyurus* recovered from roots. D) Soil populations of microbivorous nematodes.

rates was detected. Populations of microbivorous nematodes were either unchanged or increased in response to increasing rates of Clandozan 601 (Fig. 8D).

Soil pH and conductivity increased in response to increasing rates of Clandozan 601 (Fig. 9A,9B). This general response varied in magnitude according to the amount of urea added to the soil, a fact that was re-

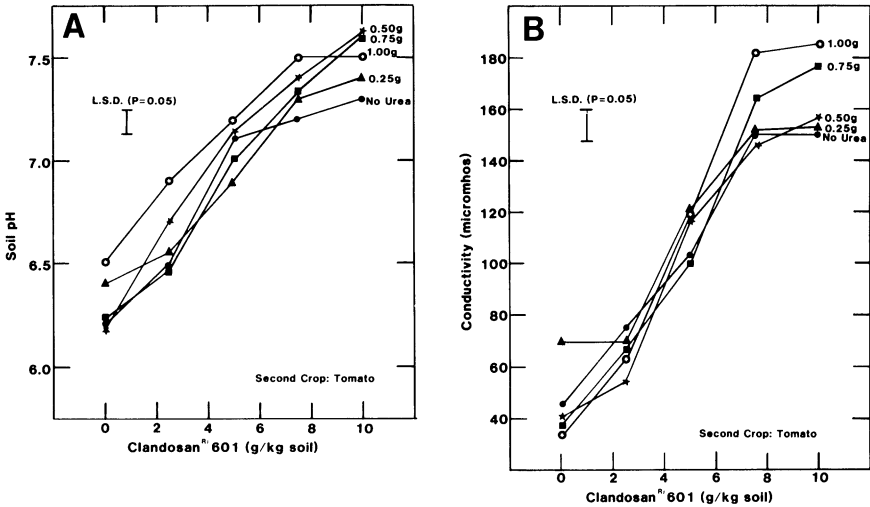


Fig. 9. Relation between soil amendment rates of Clandosan 601 and urea on soil pH (A) and electrical conductivity of soil water extract (B) following growth of tomato.

flected in significant Clandosan 601 \times urea interactions for the two variables.

Soil chitobiase activity after tomato was lower than after squash (Fig. 10A). There was no Clandosan 601 \times urea interaction on the variable. When the effect of urea on chitobiase activity was considered independently of the effect of Clandosan 601 on the variable a positive linear pattern of response to urea rates was evidenced (Fig. 5C). Conversely, when the effect of Clandosan 601 on chitobiase activity was considered independently of urea rates, a linear response to Clandosan 601 rates also was obtained, but it was negative (Fig. 5B).

Soil urease activity was highest in soils with the two highest rates of Clandosan 601 that also had the two highest rates of urea (Fig. 10B). There was a general positive response in urease activity to increasing rates of either Clandosan 601 or urea (Fig. 10C).

For soil phosphatase activity following cropping of tomato, the Clandosan 601 \times urea interaction was not significant (Fig. 11A). General patterns of response of the variable to increasing rates of urea or of Clandosan 601 were parabolic in nature (Fig. 11B). Phosphatase activity increased in response to increasing urea rates to a maximum value corresponding to 0.41 g urea/kg of soil. Phosphatase activity declined sharply with increasing rates of Clandosan 601 to a minimum corresponding to 7.79 g of Clandosan 601/kg of soil.

Neither chitobiase nor phosphatase activities in soils after cropping of tomato were correlated with numbers of galls/g of tomato root; how-

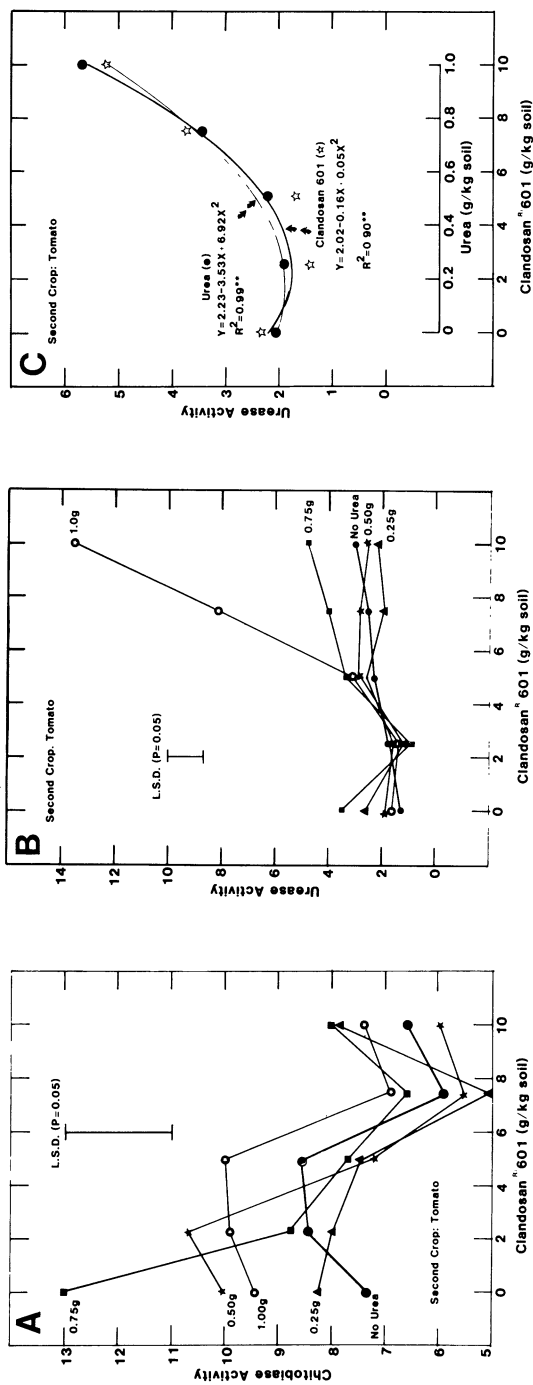


Fig. 10. Effect of amendments with Clandosan 601 and urea on soil enzymatic activities of soil planted to tomato. A) Combined effects of amendment rates on chitinase activity. B) Combined effects of amendment rates on urease activity. C) Generalized effect of Clandosan 601 or urea rates on urease activity.

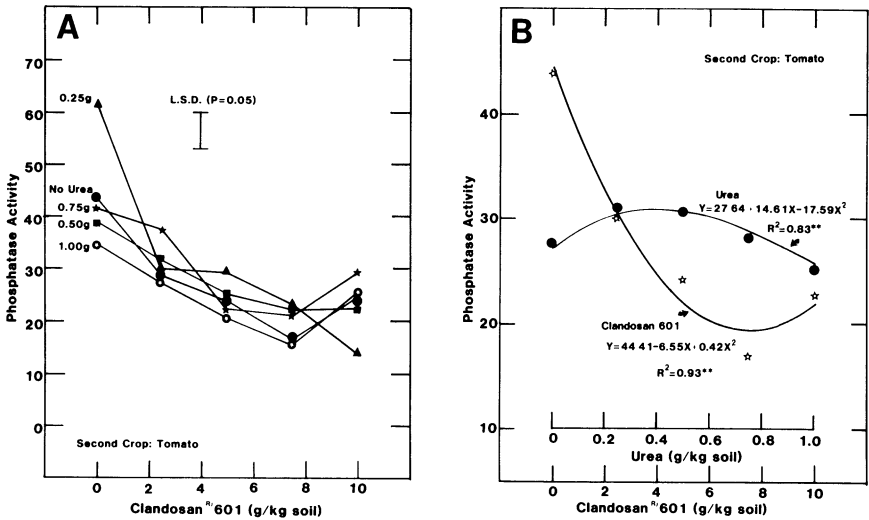


Fig. 11. Effect of Clondosan 601 and urea on acid phosphatase activity of soil with tomato. A) Combined effects of amendment rates on enzymatic activity. B) Generalized effect of Clondosan 601 and urea rates on the activity.

ever, a negative exponential relationship was evidenced between gall numbers and soil urease activity (Fig. 12).

DISCUSSION

The two chitinous materials tested in the first experiment were effective in controlling root-knot nematode. The dosages required for nematicidal activity were equivalent to those reported for purified chitin (5,6,8,16,26). The nematicidal properties of Clondosan 719 were explored by Spiegel et al. (30,31) in Israel. They reported effectiveness against root-knot and other nematodes at rates lower than those evaluated in our experiments. The soils used by those authors were neutral to alkaline in contrast to the acidic soil of our experiment; it is possible that this difference in pH may account for the differences in nematicidal rates of Clondosan 719. In neutral or alkaline soils, NH_3 would be released into the soil in response to lower rates of chitinous amendment than in acid soils where NH_4^+ would be prevalent. Our experimentation demonstrated that Clondosan 719 was slightly more effective than Clondosan 601. Clondosan 719 is a refined product obtained by treatment of Clondosan 601 with 1N HCl followed by neutralization with Na_2CO_3 (15). Treatment with HCl, while increasing the chitin content of the product, adds significantly to the cost of production. The slight increase in nematicidal activity did not justify use of Clondosan 719 over Clondosan 601.

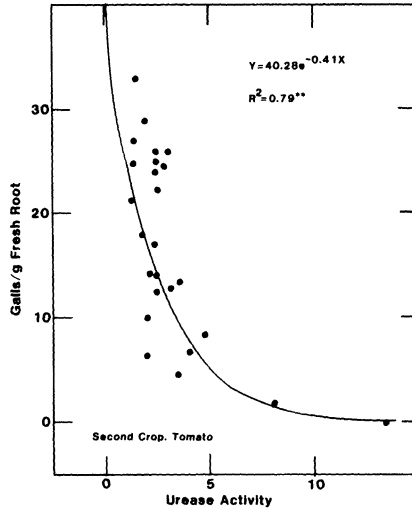


Fig. 12. Relation between number of galls induced by *Meloidogyne arenaria* in tomato roots and urease activity of soil with the crop.

Results from the first experiment demonstrated clearly that rates $\geq 1.0\%$ of Clandosan 601 were necessary to obtain adequate control of *M. arenaria* in squash and tomato roots. These rates were considered too high to be economical. The inclusion of urea in the amendment was thought desirable because of the known nematicidal effect of this inexpensive fertilizer (21). Urea at rates ≥ 300 mg/kg of soil can be nematicidal; however, at these rates the compound must be added to soil together with additional carbon sources to avoid phytotoxicity (9,23). Our results for the urea + Clandosan 601 experiment demonstrated that it is possible to obtain control of *M. arenaria* and *P. brachyurus* with several mixtures of these two materials. Acceptable levels of control of *M. arenaria* were obtained when urea was mixed 1:5 (w:w) with Clandosan 601. This mixture resulted in effective nematicidal activity at 0.3% (6.7 t/ha on a broadcast basis). This rate represents a marked improvement over the usual 40–80 t/ha reported in the literature as needed to obtain nematode control with organic amendments (13,21,25,29).

The data on enzymatic activities provide measures of microbial activities in soil (2). Urease was expected to increase in response to addition of urea to the soil if sufficient carbon was available to permit microbial degradation of the fertilizer (2,9,23). Similarly, the addition of chitinous materials to soil was expected to stimulate development of a chitinolytic microflora, hence production of chitinase and its correlative enzyme chitobiase (8,19,20,22). We chose to measure chitobiase activity rather than chitinase activity because the method for chitobiase is quicker and

more convenient than that reported for the assay of soil chitinase activity (22). Acid phosphatase activity was chosen to serve as a "control" since it is ubiquitous in soil and is produced by most microorganisms (2). Acid phosphatase activity was expected to increase in response to stimulation of the soil microflora *in toto* rather than through stimulation of any specialized fraction of it. Our results showed that treatment with Clandosan 601 + urea mixtures stimulated ureolytic and chitinolytic activities in soil. This stimulation contrasted with phosphatase activity for which no clear pattern of response to either Clandosan 601 or urea was obtained. More importantly, our data indicated significant inverse correlations between galls caused by *M. arenaria* in squash roots and both urease and chitobiase activities of soils after squash. This was in contrast to the lack of correlation observed between acid phosphatase activity and root gall numbers. Previous work has shown that addition of chitin to soil results in increased numbers of chitinolytic actinomycetes, bacteria, and fungi in soil (8,16,22,26). There are several species of fungi isolated from chitin-treated soil that are capable of destroying *Meloidogyne* eggs and some of these species are well-known pathogens of nematodes (8,18,26). We nevertheless interpret the effectiveness of Clandosan 601 + urea amendments against nematodes as not being due to the activities of any single, or even restricted numbers of microorganisms, but rather to the integrated activities of the stimulated ureolytic and chitinolytic microfloras in soil.

The enzyme data also indicated that the relation between chitinolytic microflora and nematode control was not long lived. Chitobiase activity after tomato in contrast with urease activity, was not correlated with the number of galls induced by *M. arenaria* in tomato roots. This lack of correlation suggests that stimulation of chitinolytic microflora was followed by decline in numbers of these organisms and disappearance of the enzymes produced by them. This also suggests that the pattern of response to the amendments observed in the nematode data on tomato probably was governed by the activities of the ureolytic microflora.

Data on pH and conductivity indicate little or no change in response to addition of urea to soil, but marked increases did occur in values of these variables in response to additions of Clandosan 601. This is expected since Clandosan 601 contains considerable CaCO_3 which would result in both increased pH and conductivity values when added to the acidic soil used in this study. Conductivity values also are correlated with production of ammonia and nitrates in chitin-amended soils (8). An increase in pH and conductivity may be desirable for acidic soils but not for either neutral or alkaline soils.

In conclusion, our study showed that an amendment can selectively stimulate enzymatic activities of soil microflora that will result in nematode control. The amounts of organic matter required can be con-

siderably lower than the rates reported in the literature for nonselective nematicidal organic amendments.

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