# HEMICELLULOSIC WASTE AND UREA FOR CONTROL OF PLANT PARASITIC NEMATODES: EFFECT ON SOIL ENZYME ACTIVITIES

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Combination treatments of hemicellulosic waste (HW) from paper manufacture and urea (U) were studied for their nematicidal activity and their effect on enyzmatic activities and nitrogen transformations in soil under greenhouse conditions. Urea applied at rates of 200-1000 mg N/kg soil reduced populations of plant parasitic nematodes but caused severe phytotoxicity in 'Ransom' soybeans [*Glycine max* (L) Merr.]. When HW was combined with U the effectiveness of urea against nematodes was retained but urea phytotoxicity was reduced or eliminated over the range of 200-800 mg urea-N/kg soil. Treatments with HW alone over the range of 2-32 g/kg soil reduced nematode numbers; however, the degree of reduction depended on both the nematode species and HW rate. HW alone failed to eliminate all plant parasitic nematodes but when combined with urea (400 mg N/kg soil), the combination was nematicidal at all rates tested. HW+U treatments containing 16 or 32 g HW/kg soil and 400 mg urea-N/kg soil were highly nematicidal and least phytotoxic. Application of urea to soil stimulated soil urease activity but reduced or did not affect soil invertase, phosphatase, and xylanase activities. In general, U+HW treatments resulted in higher enzymatic activities than those with urea alone. Xylanase activity was greatly stimulated by HW alone or in combination with urea. In contrast to U+HW treatments, treatments with urea alone resulted in significant accumulation of ammoniacal-N. The greatest amounts of nitrate-N were recorded in soils given U+HW treatments.

*Additional key words: Helicotylenchus dihystera, Hoplolaimus galeatus, Meloidogyne incognita, Pratylenchus brachyurus, biological control, soil enzymes, soil carbohydrases, waste management, nitrogen cycle.*

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## RESUMEN

Huebner, R.A., R. Rodíguez-Kábana y R.M. Patterson. 1983. Uso de la urea y de desperdicios hemicelulósicos para el combate de fitonematodos: efectos sobre las actividades enzimaticas del suelo. Nematropica 13:37-54.

Se estudio en el invernadero la actividad nematicida y los efectos sobre las actividades enzimáticas del suelo asi como sobre las transformaciones del nitrógeno, de

enmiendas con urea (U) y con desperdicios hemicelulósicos de la fabricación del papel (DH). Las aplicaciones de urea (U) al suelo a niveles de 200-1000 mg N/kg suelo redujeron las poblaciones de fitonematodos pero fueron fitotóxicas para la soya [*Glycine max* (L) Merr.] 'Ransom.' Los tratamientos combinados DH+U fueron eficaces contra los nematodos observándose que no causaron fitotoxicidad o bién una disminución muy marcada en la misma cuando la urea se mantuvo a niveles de 200-800 mg N/kg suelo. Los tratamientos con DH sólo, a niveles de 2-32 g/kg suelo, redujeron el número de nematodos aunque el grado de reduccón dependió de la especie y del nivel de DH utilizado. Tratamientos con DH sólo, no eliminaron completamente los nematodos pero cuando se combinó DH con urea (400 mg N/kg suelo) los tratamientos fueron nematicidas a todos los niveles de DH utilizados. Los tratamientos con DH+U que contenían DH a niveles de 16 o 32 g/kg suelo y urea a 400 mg N/kg suelo fueron muy efectivos contra los nematodos y fueron los menos fitotóxicos. La aplicación de la urea al suelo estimuló la actividad ureásica del suelo y redujo o no afectó las de la invertasa, fosfatasa y xilanasa. En general, los tratamientos DH+U resultaron en actividades enzimáticas del suelo con niveles más altos que las de los suelos tratados sólo con urea. La actividad xilanásica del suelo fué notablemente estimulada con DH sólo o en combinación con urea. La urea sola, a diferencia de los tratamientos U+DH, causó una acumulación significativa de nitrógeno amoniacal. Los suelos con mayor contenido de nitrato fueron los tratados con U+DH.

*Palabras claves adicionales: Helicotylenchus dihystera, Hoplolaimus galeatus, Meloidogyne incognita, Pratylenchus brachyurus, combate biológico, enzimas del suelo, carbohidrasas del suelo, manejo de desperdicios, ciclo del nitrógeno.*

## INTRODUCTION

The addition of organic amendments to soil to control plant parasitic nematodes has been studied extensively (14, 16, 22). Sources of organic matter used have varied, ranging from animal wastes to oil cakes, crop debris, and industrial by-products. Most effective against nematodes are materials with a high N content, or those that contain toxins or release nematicidal compounds into the soil (22). Most of the research on the use of organic amendments for nematode control has been conducted in India and other developing countries (16). Research on the subject in the United States has been relatively limited despite the availability of waste materials potentially usable for nematode control (14, 15, 19). There are at present, in the U.S.A. and other nations, serious problems related to the disposal of many industrial wastes. In the paper industry large amounts of hemicellulosic waste (HW) are generated following alkaline and bisulfite treatments of wood to release the cellulose. The HW must be disposed of in an economic manner. This paper presents results of a preliminary study to determine the effect of HW and urea on several species of plant parasitic nematodes and on some indicator variables of fertility and microbial activity of soils.

## MATERIALS AND METHODS

Hemicellulosic waste (HW) from manufacture of Kraft® paper was obtained from a paper mill near Cottonton, Alabama. The black material was allowed to dry and was then ground and sieved (1mm). The elemental composition (Table 1) was determined following standard procedures (11).

Two greenhouse experiments were conducted to study the nematicidal effects of urea and HW in soil. In experiment A, rates of urea were varied while a constant rate of HW was added to the soil; experiment B examined varying concentrations of HW at a constant rate of urea.

Soil for the experiments was a silt loam (% composition = sand 79.7, silt 12.3, clay 7.8) naturally infested with *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans-Stekhoven, *Helicotylenchus dihystera* (Cobb) Sher, *Hoplolaimus galeatus* (Cobb) Sher and *Meloidogyne incognita* (Kofoid & White) Chitwood. The soil was collected from a field at Tallassee, Alabama, with a history of both soybean and cotton culture. The soil was sieved through a 2-mm mesh screen to remove crop debris and large particles, mixed 1:1 (v/v) with sand (≤0.75 mm mesh), and divided into 1-kg quantities of moist (15%) mixture. This mixture will be referred to as soil throughout the paper.

In experiment A, aqueous solutions of urea were prepared containing 0, 20, 40, 60, 80, or 100 mg N/ml. Ten ml of the appropriate solutions were added to 1 kg quantities of the soil providing the equivalent of 0, 448, 896, 1344, 1792, or 2240 kg N/ha calculated by weight on a broadcast basis to a depth of 15 cm. The soil received urea alone or an additional constant amount of powdered HW, 32 gm/kg soil (approx. 72 MT/ha). The amendments were thoroughly and uniformly distributed in the soil by vigorous shaking in a plastic bag and transferred to 10-cm-diam, 2 liter capacity PVC pots. Each treatment was represented by eight replications (pots) arranged in a completely randomized design.

In experiment B, of identical design to experiment A, HW was added at rates of 0, 1, 2, 4, 8, 16, or 32 gm/kg soil, equivalent (broadcast basis) to 0, 2.2, 4.5, 9.0, 17.9, 35.9, or 72.0 MT/ha. Each level of HW was studied in the absence of urea and also in soil that received in addition urea at a rate of 400 mg N/kg soil.

In both experiments, the pots of treated soil were kept moist for 2 weeks before planting to allow the expected "flush" of microbial activity to subside. At the end of this period, the soil was mixed in a plastic bag and a 300 g sample was removed for nematode counts and enzyme analyses; 'Ransom' soybeans [*Glycine max* (L) Merr.] were

Table 1. Elemental composition of hemicellulosic waste (HW) matter used in the study.

| Percent of Dry Weight | | | | | | | | μg/gm Dry Weight | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | N | P | K | Ca | Mg | S | C/N | Cu | Fe | Mn | Zn |
| 14.40 | 0.424 | 0.11 | 0.09 | 8.87 | 0.41 | 1.82 | 33.96 | 80 | 5110 | 2360 | 840 |

planted (six seeds/pot) in the remaining soil. The soil sample taken for enzyme analyses was air-dried (29C) and stored in the dark at 5C until assayed. Shoot height, fresh weights of shoot and root, and populations of nematodes in the soil and roots were determined 6 weeks after planting. A portion of the final soil sample was air-dried for further enzyme analyses.

Nematode numbers were determined using the "salad bowl" technique (21) using 100 $cm^3$ soil for soil populations or with the entire recovered root system for root populations. Populations were expressed as no./100 $cm^3$ soil or no./g fresh root, respectively.

Soil pH was measured with a Corning® Model 12 pH meter using 10 g of air-dried soil mixed with 10 ml $H_2O$ (13). For determination of nitrate-nitrogen, an additional 10 ml $H_2O$ was mixed with the soil suspension used for pH determination and 10 ml of the diluted suspension was centrifuged (4000 *g*, 20 min). Two ml of the clear supernatant was analyzed for nitrate nitrogen by the phenyldisulfonic acid method (13). Ammoniacal nitrogen was determined by shaking 5 g soil with 20 ml 10% (w/w) acidified NaCl (pH 2.5) for 30 min, centrifuging 10 ml of the suspension (4000 *g*, 20 min), and analysing the amount of $NH_4$–N in 1 ml of supernatant following a Nessler method (1).

Urease (urea amidohydrolase, E.C. 3.5.1.5) activity in the soil was measured [substrate = 10% (w/v) urea] using the method of Rodriguez-Kabana and King (19); activity was expressed as μg of $NH_4$–N released per hr per g of soil.

Xylanase (xylan 4-xylanohydrolase, E.C. 3.2.1.8) was determined [substrate = 1% (w/v) xylan suspension] following a modification of Sorensen's method (24) described by Rodriguez-Kabana (18); activity was expressed as μg xylose produced per hr per g of soil.

Invertase (beta-fructofuranoside fructohydrolase, E.C. 3.2.1.26) activity was determined by a modification of Hofmann's method (10). Ten ml of 5% (w/v) sucrose was added to 10 g of air-dried soil. The suspension was stirred and placed in an incubator for 3 hr at 37C. After incubation, 40 ml of $H_2O$ was added and after mixing, 10 ml of the suspension was centrifuged (4000 *g*, 20 min). The reducing sugar content of 1 ml of the clear supernatant was determined by Nelson's colorimetric

procedure (17); invertase activity was expressed as $\mu$g of reducing sugar (dextrose standard) released per hr per g of soil.

Aryl phosphatase (orthophosphoric monoester phosphahydrolase, E.C. 3.1.3) activity in soils was determined (substrate = 6.75 g/l phenyl disodium phosphate) following the method of Hofmann (10); activity was expressed as $\mu$g of phenol released per hr per g of soil.

All data were analyzed following standard procedures for analysis of variance; differences between means were evaluated for significance using a modification of Duncan's multiple range test (26). Unless otherwise indicated differences referred to in the text were significant at the 5% or lower level of probability.

## RESULTS

*Effect of varying rates of urea alone or with a constant level of hemicellulosic waste (Experiment A).*

Numbers of plant parasitic nematodes generally declined in response to additions of urea (Table 2) both in the presence of HW (U+HW-series) and in its absence (U-series). Data in Table 2 are preplant values, and therefore reflect the effect of urea or HW without the influence of plant growth. At the end of the experiment, *H. dihystera, H. galeatus,* and *M. incognita* were absent from all soil samples but they were present in the roots of all plants where urea-N was applied at concentrations below 400 mg/kg soil (Table 3). *H. galeatus* and *H. dihystera* were more numerous in roots from control soil in the U-series than in those from the control of the U+HW series which contained only 32 g HW/kg soil.

In the U series, the number of surviving plants was severely reduced following applications of urea; however, in the U+HW series, urea had no deleterious effect on seedling survival at rates below 600 mg N/kg soil (Table 4). Urea had no significant effect on plant height in either series of treatments except when applied at rates higher than 600 mg N/kg soil. Soybeans were taller in the U+HW series than in the U-series for all levels of urea-N below 800 mg/kg soil. With only one exception (600 mg N treatment), all urea treatments in the U-series reduced plant weight, but in the U+HW series this was true only at the 2 highest urea levels.

Soil pH was higher in soils that received HW in addition to urea than in those with urea alone (Fig. 1A). In the U-series, increasing concentrations of urea resulted in increased pH at the first sampling; however, this response was not true at the final sampling. The pH of soils

Table 2. Numbers of plant parasitic nematodes in 100 $cm^3$ soil taken at planting time from soil amended with increasing rates of urea (U) with or without a constant rate (32 g/kg soil) of hemicellulosic waste (HW).[a]

| Urea-N (mg/kg soil) | *Hoplolaimus galeatus* | | *Meloidogyne incognita* | | *Helicotylenchus dihystera* | |
|---|---|---|---|---|---|---|
| | U | U+HW | U | U+HW | U | U+HW |
| 0 | 5.0 a | 6.9 a | 4.0 a | 19.0 a | 3.7 b | 4.5 a |
| 200 | 2.0 b | 0.7 b | 3.2 a | 2.4 b | 7.2 a | 2.5 ab |
| 400 | 0.0 c | 0.0 b | 0.0 a | 0.0 b | 2.2 bc | 1.2 b |
| 600 | 0.0 c | 0.0 b | 0.0 a | 0.0 b | 0.1 c | 0.2 b |
| 800 | 0.0 c | 0.0 b | 0.0 a | 0.0 b | 0.1 c | 0.2 b |
| 1000 | 0.0 c | 0.0 b | 0.0 a | 0.0 b | 0.0 c | 0.0 b |

[a]Values are the averages of 8 replications; values within the same column followed by a common letter were not statistically different (P=0.05).

Table 3. Numbers of plant parasitic nematodes per gm of roots from plants grown in soil amended with increasing rates of urea (U) with and without a constant rate (32 g/kg soil) of hemicellulosic waste (HW).[a]

| Urea-N (mk/kg soil) | *Hoplolaimus galeatus* | | *Meloidogyne incognita* | | *Helicotylenchus dihystera* | |
|---|---|---|---|---|---|---|
| | U | U+HW | U | U+HW | U | U+HW |
| 0 | 17.4 a | 1.2 a | 1.2 a | 10.4 a | 94.6 a | 20.3 a |
| 200 | 1.3 b | 3.3 a | 0.0 a | 0.3 b | 7.1 b | 25.9 a |
| 400 | 0.0 b | 1.4 a | 0.0 a | 0.0 b | 0.0 b | 9.1 a |
| 600 | 0.0 b | 0.0 a | 0.0 a | 0.0 b | 0.0 b | 0.2 a |
| 800 | 0.0 b | 0.0 a | 0.0 a | 0.0 b | 0.0 b | 0.0 a |
| 1000 | 0.0 b | — | 0.0 a | — | 0.0 b | — |

[a]Values are the averages of 8 replications; values within the same column followed by a common letter were not statistically different (P=0.05).

in both series of treatments was generally lower at the final sampling than at the time of planting.

Soil at planting time from treatments with urea contained more $NH_4^+$–N than at the final sampling (Fig. 1B). The initial ammoniacal N content was generally greater with increasing amounts of urea in

Table 4. Numbers of plants, shoot height and shoot weight of soybeans grown in soil amended with increasing rates of urea (U) with and without a constant rate (32 g/kg soil) of hemicellulosic waste (HW).[x]

| Urea-N (mg/kg soil) | Plants per pot[y] | | Height (cm) | | Weight (gm) | |
|---|---|---|---|---|---|---|
| | U | U+HW | U | U+HW | U | U+HW |
| 0 | 4.63 a | 4.13 a | 16.31 a | 19.19 a | 1.04 ab | 1.38 ab |
| 200 | 2.63 b | 3.63 a | 12.56 a | 18.79 a | 0.74 c | 1.63 a |
| 400 | 0.88 b | 4.50 a | 15.33 a | 17.51 a | 0.69 c | 1.27 bc |
| 600 | 0.88 b | 1.38 b | 15.70 a | 18.23 a | 1.24 a | 1.59 ab |
| 800 | 0.38 b | 1.38 b | 12.67 a | 12.58 b | 0.53 c | 1.02 c |
| 1000 | 0.25 b | 0 | 16.45 a | 0 | 0.46 c | 0 |

[x]Values are the averages of 8 replications; values within the same column followed by a common letter were not statistically different (P=0.05).
[y]Six seeds were planted per pot.

both series of treatments. This trend was also true for final samples from the U-series; final samples from the U+HW series contained very low amounts of $NH_4^+$–N.

Urea-treated soil contained more nitrate N at the end of the experiment than at the first sampling (Fig. 1C). At the final sampling, treatments in the U+HW series with urea at rates above 200 mg/kg soil contained more nitrate N than corresponding treatments with urea alone.

The addition of HW resulted in a significant increase in urease activity at all urea application levels (Fig. 2A). Within each series at both sampling times, urease activity generally increased with increasing concentration of urea-N. Also, for both series of treatments, the final soil samples contained higher urease activity than planting-time samples.

Xylanase activity of soils was stimulated by the addition of HW (Fig. 2B). This was particularly evident at the final sampling time when soils in the U+HW series were 7-29 times more active than those from the U-series. At the first sampling time, only soils from the U+HW series with no urea or those with 200 or 1000 mg N/kg soil evidenced higher xylanase activity than those of corresponding treatments of the U-series.

Invertase activity increased over the course of the experiment (Fig. 2C). Also, at the final sampling, soils with urea from the U+HW series had higher invertase activity than those in the U-series; the reverse was true for the first sampling. Increasing amounts of urea resulted in significant reductions in invertase activity of soils in the U-series at both

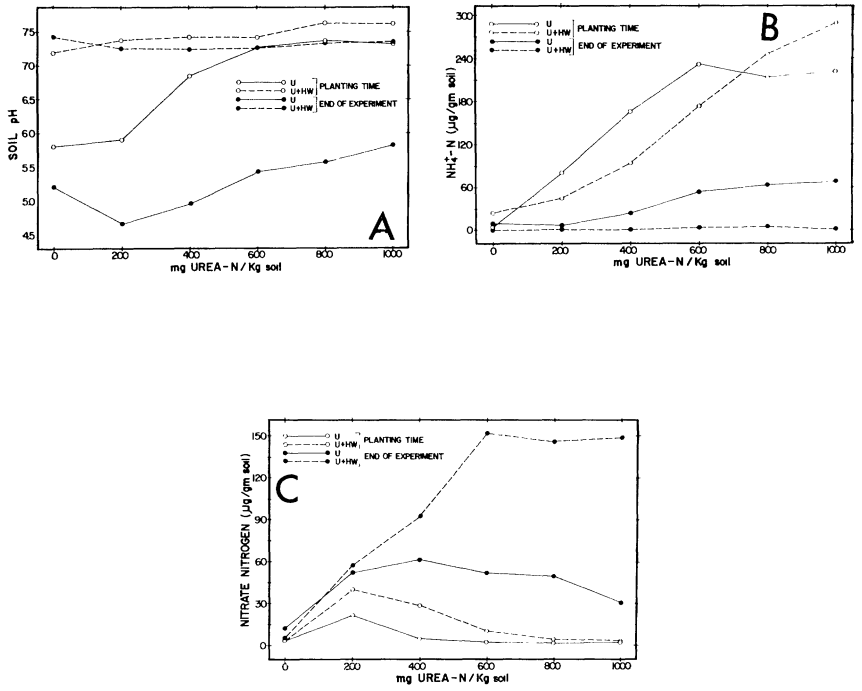


Fig. 1. Effect of application to soil of increasing rates of urea (U), with or without a constant rate (32 gm/kg soil) of hemicellulosic waste (HW), on soil pH and 2 nitrogen cycle variables, at two different sampling times.

sampling times. Urea at rates of 400 mg N/kg soil or higher reduced invertase activity at the first sampling in the U+HW series; at the final sampling, invertase activity was lower than the control only in soils containing the 2 highest rates of urea.

Aryl phosphatase activity was higher in soils with urea from the U+HW series than in soils from corresponding treatments in the U-series (Fig. 2D) Aryl phosphatase activity was higher at the first sampling than at the final sampling for both series of treatments. Increasing urea concentrations resulted in reduced aryl phosphatase activity in the U-series. The same was true for the U+HW series but the reductions were not as pronounced and were not significant at the final sampling at the 3 highest urea rates.

*Effect of increasing levels of hemicellulosic waste with a constant urea rate (Experiment B).*

Numbers of *H. galeatus* in samples taken at planting time declined with increasing levels of HW above 2/kg soil in treatments with no

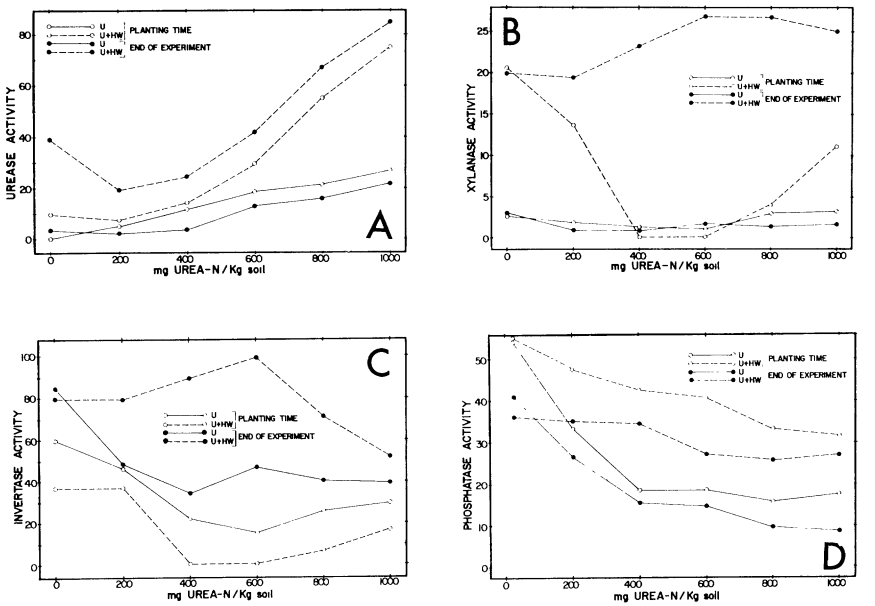


Fig. 2. Effect of application to soil of increasing rates of urea (U), with or without a constant rate (32 gm/kg soil) of hemicellulosic waste (HW), on soil enzymatic activities, at two different sampling times.

urea (HW-series); other nematode species in the samples were present in numbers too low for reliable assessment (Table 5). The addition of urea (HW+U-series) resulted in a drastic reduction in numbers of *H. galeatus;* however, there were no significant differences between treatments in the HW+U-series.

At the final sampling, the only nematode in signficant numbers in the soil was *H. galeatus* and only in the HW series. Soil samples from the HW+U-series contained very few plant parasitic nematodes. There were lower numbers of nematodes in roots from the HW+U series than in those from treatments with no urea (Table 6). All levels of the amendment in the HW-series reduced numbers of *H. galeatus* and *P. brachyurus* in the roots but had no effect on larvae of *M. incognita;* no pattern of response to HW could be established for *H. dihystera* in roots from the HW-series soils.

There were no differences in numbers of soybean plants in soils from the HW-series (Table 7); however, a phytotoxic effect was observed when urea was included in the treatments. Plant numbers decreased in the HW+U-series compared with the HW-series, except at amendment levels greater than 8 g/kg soil. Plant height was increased by the amend-

Table 5. Number of *Hoplolaimus galeatus* in 100 cm³ soil taken at planting time from soil amended with increasing rates of hemicellulosic waste (HW) with and without a constant rate (400 mg N/kg soil) of urea (U).[x]

| HW rate (g/kg soil) | Planting time[y] | | End of Experiment | |
|---|---|---|---|---|
| | HW | HW+U | HW | HW+U |
| 0 | 26.9 b | 1.75 a | 8.63 a | 0.00 a |
| 2 | 51.6 a | 2.25 a | 4.24 a | 0.00 a |
| 4 | 15.4 c | 2.25 a | 5.13 a | 0.00 a |
| 8 | 9.5 c | 4.38 a | 7.50 a | 0.00 a |
| 16 | 9.5 c | 0.75 a | 3.13 a | 0.00 a |
| 32 | 11.6 c | 0.75 a | 4.00 a | 0.50 a |

[x]Treatments were added to soil 2 weeks prior to planting.
[y]Values for variables are the averages of 8 replications; values within the same column followed by a common letter were not statistically different (P=0.05).

Table 6. Numbers of plant parasitic nematodes per g of root from soybeans grown in soil amended with increasing concentrations of hemicellulosic waste (HW) from paper manufacture and a constant rate (400 mg N/kg soil) of urea (U).[x]

| HW Rate (g/kg soil) | *H. galeatus* | | *P. brachyurus* | | *M. incognita* | | *H. dihystera* | |
|---|---|---|---|---|---|---|---|---|
| | HW | HW+U | HW | HW+U | HW | HW+U | HW | HW+U |
| 0 | 113.6 a | 0.0 a | 15.5 a | 0.0 a | 9.4 a | 0.0 a | 25.6 a | 0.0 a |
| 2 | 41.7 bcd | 5.0 a | 4.3 cd | 4.7 a | 8.1 a | 0.0 a | 12.8 bc | 5.0 a |
| 4 | 61.5 b | 0.0 a | 9.0 bc | 3.6 a | 8.9 a | 0.0 a | 27.9 a | 4.4 a |
| 8 | 32.0 cd | 0.1 a | 5.6 cd | 2.6 a | 9.8 a | 0.0 a | 9.9 c | 2.7 a |
| 16 | 28.3 cd | 1.7 a | 10.6 b | 2.8 a | 2.9 a | 0.0 a | 20.3 abc | 5.0 a |
| 32 | 19.1 d | 8.7 a | 3.9 d | 3.6 a | 3.5 a | 0.0 a | 10.2 c | 7.0 a |

[x]Figures for variables are the averages of 8 replications; those within the same column followed by a common letter were not statistically different (P=0.05).

ment in the HW-series but urea decreased plant height at each level of HW. Shoot weights followed the trend described for plant numbers; plants in the HW+U-series weighed less than the corresponding plants of the HW-series.

Soil at planting time had a higher pH than at the final sampling time (Fig. 3A). At the end of the experiment, soils in the HW+U-series had lower pH values than those without urea. At the first sampling, soils without urea had lower pH than those with the fertilizer except

Table 7. Numbers, shoot height and shoot weight of soybeans grown in soil amended with increasing concentrations of hemicellulosic waste (HW) from paper manufacture and a constant rate (400 mg N/kg soil) of urea (U).[a]

| HW Rate (g/kg soil) | Plants per pot[y] | | Height (cm) | | Weight (g) | |
|---|---|---|---|---|---|---|
| | HW | HW+U | HW | HW+U | HW | HW+U |
| 0 | 5.13 a | 1.38 d | 20.69 c | 10.14 c | 1.34 b | 0.49 d |
| 2 | 4.13 a | 3.13 bc | 30.58 a | 13.05 bc | 2.41 a | 1.02 c |
| 4 | 5.13 a | 2.75 bc | 27.56 ab | 18.69 a | 1.87 ab | 1.73 a |
| 8 | 4.00 a | 1.37 d | 28.86 ab | 13.05 bc | 2.11 a | 1.35 abc |
| 16 | 4.00 a | 4.00 ab | 29.93 ab | 19.73 a | 2.18 a | 1.61 ab |
| 32 | 3.75 a | 5.25 a | 28.18 ab | 18.30 a | 2.07 a | 1.12 bc |

[a]Values are the averages of 8 replications; those within the same column followed by a common letter were not statistically different (P=0.05).
[y]Six plants were planted per pot.

for soils with the 2 highest levels of HW which did not differ in pH. The addition of HW resulted in increased soil pH, a response directly dependent on the amount of the amendment; this relation was more clearly seen at the final sampling time than at planting.

Soil samples at planting time contained more ammoniacal N than at the end of the experiment (Fig. 3B). There were no differences between treatments with respect to $NH_4^+$–N content at the final sampling; however, at the first sampling, soils from the HW+U-series contained more $NH_4^+$–N than those from the HW series.

Highest nitrate content was determined in final samples from the HW+U-series (Fig. 3C). In those samples more nitrate was found in soils with the 3 highest levels of HW than in soils with less of the amendment. The lowest nitrate content was found in samples from the HW-series. Although soils in the HW+U-series at the first sampling contained significant amounts of nitrate N, there was no sharp response to concentration of HW as was observed for the same series of treatments at the final sampling.

Urease activity of soils increased in response to increasing rates of HW in both series of treatments (Fig. 4A). The only exception to this pattern was in the HW+U-series where HW was present at 32 g/kg soil. Urease activity at the first sampling was higher in the HW+U-series than in the HW-series; however, there were no differences between the two series at the final sampling except for the highest rate of HW

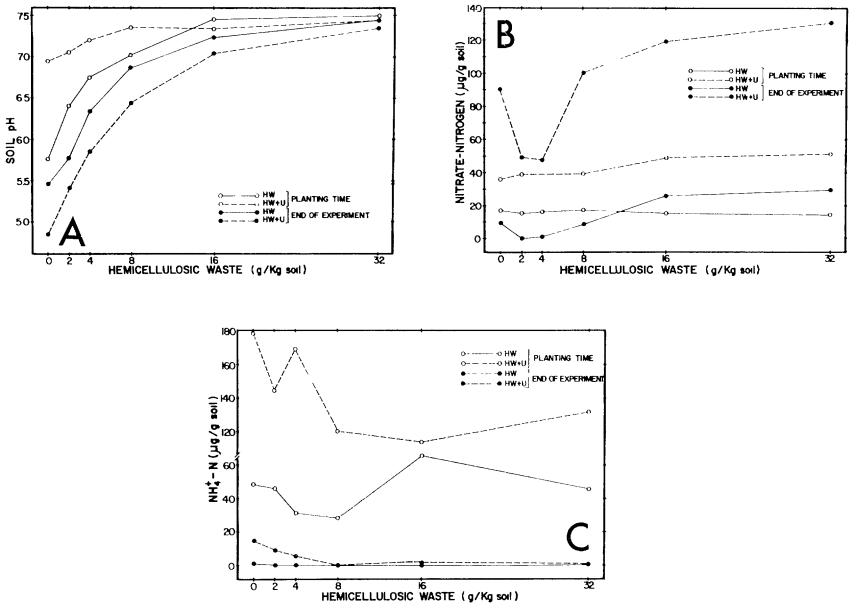


Fig. 3. Changes in soil pH, nitrate and ammoniacal nitrogen in response to applications to soil of increasing rates of hemicellulosic waste (HW), with or without a constant rate (400 mg N/kg soil) of urea (U), at two different sampling times.

in which the soils of the HW-series had more urease activity than those of the HW+U-series.

At the final sampling, xylanase activity of soils from both series of treatments increased almost linearly in response to increasing amounts of HW (Fig. 4B); differences in activity between treatments were not significant. At the first sampling, the soils in the HW+U- series evidenced little or no xylanase activity but soils in the HW series exhibited activity proportional to the amount of the amendment in the treatment.

Invertase activity in soils of the HW+U-series from the first sampling decreased with increasing amount of HW so that soils with the 3 highest levels of the amendment evidenced little or no activity (Fig. 4C). In contrast, at the same sampling, all soils in the HW series had considerable invertase activity and there was only slight decline in this activity in response to increasing HW concentration. At the final sampling invertase activity values in soils of the HW+U-series increased rapidly in response to HW levels up to 8 g/kg soil, followed by a slight decline in response to the 2 highest concentrations of HW. Soils that received the 3 highest amendment rates in the HW-series exhibited

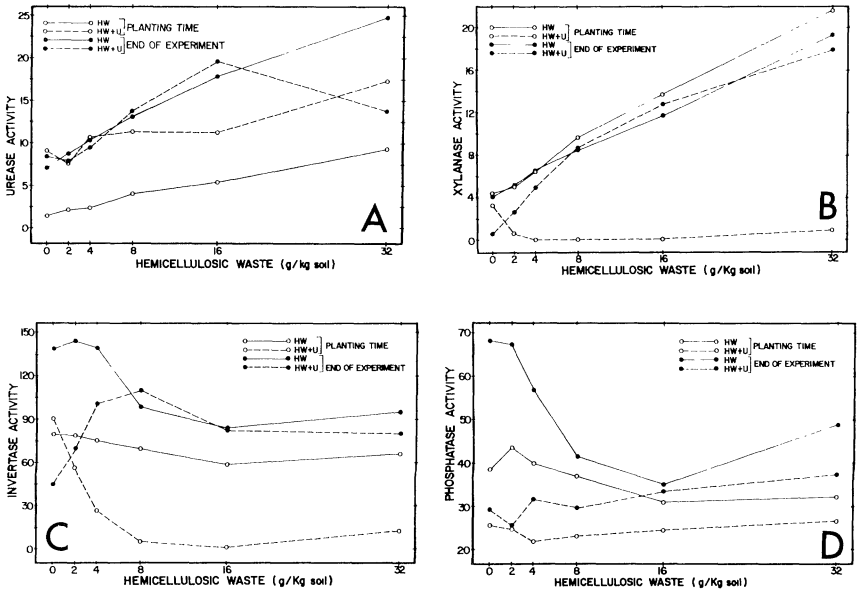


Fig. 4. Effect of applications to soil of increasing rates of hemicellulosic waste (HW), with or without a constant rate (400 mg N/kg soil) of urea (U), on soil enzymatic activities, at two different sampling times.

lower invertase activity at the final sampling than the other treatments of the series; however, at this sampling, invertase activity in soils of the series was always either equal or higher than soils of corresponding treatments in the HW+U-series.

Aryl phosphatase activity was higher in soil of the HW-series than in soil of the HW+U series (Fig. 4D). Levels of enzymatic activity were higher at the final sampling than at the first sampling. Increasing the rate of HW in the soil resulted in a drastic reduction in the activity for soils in the HW series; soils in the HW+U-series either showed no change in activity or a slight increase in response to increasing rates of HW.

## DISCUSSION

As urea is added to the soil it is converted through the action of urease to the ammonium ion and $CO_2$ according to the following equation:

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{urease}} (NH_4)_2CO_3 + H_2O \rightarrow 2NH_4OH + CO_2$$

The ammonium ion is in equilibrium with free ammonia and soil pH determines the direction of the equilibrium (7,9).

$$NH_3 + H_2O \rightleftharpoons NH_4 + OH^-$$

Since soil pH is raised by the release of $OH^-$ ions, the equilibrium is shifted to the left and ammonia gas is produced. Cell membranes are relatively impermeable to the ammonium ion, but free ammonia enters easily (27). High concentrations of ammonia have been shown to reduce seed germination, affect seedling growth (2, 6, 12), and kill nematodes (8, 20). Our results thus confirm the finding that urea is an effective nematicide and that incorporation of a carbon source with urea reduces phytotoxic effects from accumulation of ammonia and nitrites (19).

Ammoniacal-N in soil is converted to nitrite and nitrate in the two-step process of nitrification. First, $NH_4$ is converted to nitrite principally by *Nitrosomonas* spp. and, concomitantly, nitrite is transformed to nitrate by *Nitrobacter* spp. Normally, there is no accumulation of nitrite and the process results in formation of nitrate. However, high levels of ammonia are inhibitory to *Nitrobacter* spp. and result in the accumulation of nitrites in soil (5). HW added in combination with urea in our experiments thus served as a "safener" preventing accumulation of phytotoxic nitrites.

Soil enzymatic activities can be viewed as an index of biological activity of the soil (4). Data for enzyme activities and other variables studied in the experiment permit us to assess the effects of the amendments on microbial activities in the soil and the relation of these activities to nematodes and plants.

In the experiment with a constant HW rate, plant vigor and nematode numbers were severely reduced in the U-series, even at the lowest rate of urea. The addition of HW further reduced nematode numbers but also reduced the phytotoxic effect of urea at levels below 600 mg urea-N/kg soil. HW apparently provided a ready source of carbon permitting microorganisms in the soil to metabolize the nitrogen added as urea and convert it into protein (enzymes) and other nitrogenous compounds, thereby reducing accumulation of nitrite and phytotoxicity. A reflection of this set of events was the increase in urease activity observed after the addition of HW in the U+HW-series. Urease activity is known to increase following the addition of organic amendments (3). The amount of HW added in experiment A did not provide sufficient carbon to metabolize all of the nitrogen when urea was added at rates greater than 600 mg N/kg soil. This resulted in accumulation of ammonia causing phytotoxicity and a consequent decline in enzymatic activity and nematode numbers. The decrease in activities of other enzymes at the first sampling also can be attributed to this toxic effect.

Of particular interest in the experiment with a constant HW rate were the results obtained for xylanase activity. Soil xylanase is an adaptive enzyme which accumulates in response to the amount of xylan present in the soil (18,24,25). It is thus not surprising that in the U-series where no HW (xylans) was present, xylanase activity did not increase. Conversely, in treatments where HW was present, the values of xylanase activity were significantly higher than those in the U-series. Results also indicate that, at least initially, the presence of high amounts of urea in the U+HW-series may have resulted in restriction of growth of xylanase-producing microorganisms. This effect was probably due to the toxicity of released ammonia. However, the effect of urea was short-lived since, by the end of the experiment, xylanase activity in the U+HW-series had increased, indicating a recovery in the numbers of xylanase-producing microorganisms. The proposed interpretation of urea effects on xylanase activity is corroborated by similar effects observed for invertase activity which is known to be directly related to microbial activity and numbers (4, 23).

Phosphatase activity is also known to increase with the addition of organic amendments or following moderate fertilization; however, activity decreases with high doses of fertilizer (4,23). This agrees with our resutls since phosphatase activity decreased in response to higher rates of urea; however, the decline was not as sharp in the U+HW-series as in the U-series.

The experiment with a constant urea rate showed that HW also had nematicidal properties and further confirmed that the addition of HW reduced phytotoxicity caused by urea. As expected, low numbers of plant parasitic nematodes were found in soil and roots of treatments containing urea. Plant parasitic nematodes were found at all levels of HW in the HW-series, but there was a general decline in numbers as the concentration of HW was increased.

Plant response was reduced when urea was added at HW levels below 4-8 g/kg soil; however, as rates of HW increased, plant response improved, presumably due to increased carbon availability for assimilation of nitrogen. Ammoniacal-nitrogen content was low in soils in the HW-series and decreased in the HW+U-series at HW levels above 4 g/kg soil, indicating the onset of nitrification.

Using enzymatic activity as an index, microbial populations also were affected by the amendments; thus urease activity increased in the HW+U-series as the HW concentration increased. Invertase activity had increased at the end of the experiment, though activity in the HW+U-series was not as high as in the HW-series, probably due to an excessive amount of urea. Xylanase and phosphatase activities in the experiment

paralleled that of invertase activity. The increase in enzymatic activity observed in response to increasing amounts of HW in the HW+U-series is interpreted as reflecting increased microbial activity and the availability of progressively higher amounts of carbon for the metabolism of nitrogen.

From the two experiments, there appeared to be an "all or none" effect of urea at the rates used. The 400 mg rate used in one experiment was excessive when used alone or with low amounts of HW. Evidently, lower rates of urea need to be studied to determine the most efficient nematicidal rate in combination with HW that would result in no phytotoxicity. The efficacy of urea might be increased through the use of nitrification inhibitors which would prevent the accumulation of nitrite.

The mode of action of HW probably involves a stimulation of activity of fungi and bacteria which in turn results in reduction of plant parasitic nematode numbers. Although HW at a constant rate of 32 g/kg soil was used in one experiment, it appears from the other experiment that 16 g/kg soil is also stimulatory. Since this method of control is being designed for home gardeners, a lower rate would reduce the labor required for incorporation into the soil. Amending soil with urea and HW has potential as an inexpensive and effective method for nematode control. Further tests on rates are needed and field tests are underway to test the practicality under natural conditions.

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