

USE OF MIXTURES OF UREA AND BLACKSTRAP MOLASSES FOR CONTROL OF ROOT-KNOT NEMATODES IN SOIL [USO DE MEZCLAS DE UREA Y MELAZA DE CAÑA PARA COMBATIR NEMATODOS NODULADORES EN EL SUELO]. R. Rodríguez-Kábana and P. S. King, Department of Botany, Plant Pathology, and Microbiology, Agricultural Experiment Station, Auburn University, Auburn, AL 36830, U.S.A.

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ABSTRACT

Additions of urea to soil at rates of 0.4 g/kg soil or higher resulted in significant reductions in numbers of galls per g of fresh root of squash (*Cucurbita pepo*) caused by *Meloidogyne arenaria*. These rates of urea resulted in significant accumulations of nitrate and ammoniacal N in soil, increased conductivity of soil water extract and phytotoxicity to squash plants. Application of mixtures of blackstrap molasses with urea to soil resulted in improved control of *M. arenaria*, no significant accumulation of nitrate or ammoniacal nitrogen and no phytotoxicity to plants. The molasses + urea treatments resulted in increased urease and invertase activity of the soil and, for concentrations of urea of 0.4 and 0.8 g/kg soil, in increased numbers of microbivorous nematodes.

Key words: soil fertility, nematode control, root-knot nematodes, C/N ratio.

INTRODUCTION

The addition of organic manures to soil can have a profound effect on populations of plant parasitic and other nematodes. Organic matter amendments have been reported repeatedly to reduce nematode populations in soil (7,8,9,10,12,15,19). Reports in the literature indicate that amendments containing high levels of ammoniacal N or that can result in the liberation of NH_3 upon addition to soil are particularly effective in reducing nematode populations in soil (3,4,11,18,20). Utilization of this knowledge has been limited because of standardization of amendment application to soil is practically impossible; the amount and type of N in the amendment vary with the source. In addition, the amounts of amendments per ha required for effectiveness are generally very high so that concentrations of 1% (w/w) or higher of the amendment in the soil must be attained before significant reductions in nematode numbers are observed. Addition to soil of fertilizers containing ammoniacal N has occasionally been reported effective against plant parasitic nematodes (3,4,11,20). Although the use of fertilizers would eliminate standardization problems posed by organic amendments, there is little information available on dosages required or on the manner in which these materials should be used to obtain consistent nematocidal activity.

This paper reports results of a study to determine the nematocidal efficacy of a nitrogenous fertilizer (urea) alone and in combination with a standardized carbon source (black strap molasses).

MATERIALS AND METHODS

Soil for the experiment was a sandy loam infested with *Meloidogyne arenaria*, with

a pH of 6.5 and an organic matter content of less than 1%. The moist (50% field capacity) soil was sieved (2 mm) and apportioned in 500-gm quantities into individual plastic bags. Ten ml of the appropriate solution (or water) was thoroughly mixed with the contents of each bag which was then transferred into 1-l pots of 10 cm diam. The pots were maintained in a moist condition in a greenhouse and, after 2 weeks, 5 yellow crookneck squash (*Cucurbita pepo*) seeds were planted into each pot to serve as root-knot nematode indicators. After six weeks of growth, the plants from each pot were carefully separated from the soil, the roots washed, and the number of galls caused by *M. arenaria* were counted. The fresh weight of shoots and roots, and the height of each plant were also recorded. Fifty cm³ of soil from each pot was collected for extraction of nematodes and the remaining soil was spread on a table and allowed to dry at 25 C. The dried soil was then transferred to glass bottles and stored in the dark at 5 C until analyzed.

A greenhouse experiment was designed to test the effect of urea, and urea + a carbon source on nematodes. A series of solutions containing increasing amounts of urea was prepared by dissolving 0, 1.25, 2.5, 5, 10, or 20 gm of the chemical in 30 ml of demineralized water in 250-ml volumetric flasks prepared in duplicate. One flask received 200 ml of a stock aqueous solution of black strap molasses (200 ml of black strap molasses [sp. gr. 1.300] + 800 ml of water) and the other flask the same volume of water only. The flasks were then brought to the mark with water and the contents were mixed well before use. When 10 ml of these solutions was added to 500 gm of soil it provided 0, 0.2, 0.4, 0.8, or 1.6 g urea/kg soil, respectively.

Nematode Analysis - Nematodes were extracted from soil by a modified flotation-sieving technique in which a molasses solution was used as the suspending medium (16). The nematodes extracted were counted using a dissecting stereoscope.

Chemical Analysis - Invertase activity was determined by a modification of the method described by Hofmann (5). Ten ml of a 5% (w/w) aqueous sucrose solution was added to 5 gm of air-dried soil in a 30-ml-square specimen bottle, and the bottle stoppered and incubated at 40° C for 4 hrs. Twenty ml of water was added to the bottle and mixed well. Bottles with water and soil instead of sucrose were also included as controls. A 10-ml aliquot of the suspension was transferred to a 12-ml test tube and centrifuged for 20 min at 4500 g using an IH model centrifuge. One ml of the clear supernatant from the tube was then used to determine reducing sugars with the method of Nelson (13). Invertase activity was expressed in terms of ug of reducing sugars (glucose = standard) produced per hour per gram of soil.

Urease activity was determined with a modification of Hofmann's method (5) by adding 10 ml of 10% (w/w) aqueous urea solution to 5 gm of air-dried soil in a 60-ml-square bottle; soil and water in a bottle served as a control. The bottles were stoppered and incubated for 5 hr at 40° C, then each received 20 ml of acidified 10% NaCl solution (pH 2.5 with 0.1 N HCl) followed by 20 ml of water. The bottles were shaken and allowed to stand for 15 min to settle soil particles. For each bottle 1 ml of the clear supernatant was placed in the middle well of a 69-mm-diam Obrick (14) microdiffusion dish containing 4.5 ml of 40% (w/w) K₂CO₃ in the outer well, 1 ml of the K₂CO₃ solution in the middle well, and 2 ml 0.1 N HCl in the center well. The dish was closed with the lid and, after mixing of the contents in the middle well, distillation was allowed to proceed for 16 hr. One ml from the center well was then used to determine ammoniacal nitrogen using a Nessler method (1). Urease activity was expressed as ug of ammoniacal N released/hr/gm soil.

Ammoniacal N was extracted from 2 g of dry soil by adding 20 ml of acidified 10% NaCl (pH 2.5) into a 125 ml Erlenmeyer flask with 2 g of soil. The mixture was shaken for 30 min, and 20 ml of water was added and mixed. Ten ml of soil suspension was

centrifuged as described for invertase activity and 1 ml of the clear supernatant was used to determine ammoniacal N by Nesslerization (1,6).

Nitrate nitrogen in 5 gm of dry soil was determined by the phenyldisulphonic acid method described by Jackson (6) using 40 ml of demineralized water to extract the salts.

Soil pH of a suspension prepared by mixing 5 gm of dry soil in 10 ml of water was determined with a pH meter. Conductivity of water extract was determined by shaking 5 gm of soil and 20 ml of demineralized water in a 125-ml Erlenmeyer flask for 30 min. A 10-ml aliquot of the soil suspension was then centrifuged as described before and conductivity of the supernatant was determined using a conductivity cell (K=1) connected to an Industrial Instruments, Model RC 16 B2 conductivity bridge.

Statistical Analysis - All data were analyzed following standard procedures for analysis of variance and the least significant difference between means calculated (17). Unless otherwise specified, all differences mentioned in the text were significant to the 5% or lower level of probability.

RESULTS

The number of galls per gram of fresh root (GPR) was reduced in plants grown in soil treated with concentrations of urea of 0.4 g/kg soil or higher, irrespective of the presence or absence of molasses in the soil (Fig. 1A). GPR values for the 0 and the lowest concentration of urea were significantly lower in pots that received molasses than in those without it.

The number of larvae of *M. arenaria* in soil increased in response to the lowest concentration of urea and declined to levels significantly below the control when the amount of urea in the soil was 0.4 g/kg soil or higher (Fig. 1B). The presence of molasses in the treatment resulted in increased numbers of larvae with the lowest concentration of urea but did not affect the numbers in the other treatments.

The effect of urea on microbivorous nematodes was not significant (Fig. 1C); however, the inclusion of molasses in the treatments resulted in marked increases in numbers of these nematodes for urea concentrations of 0.4 and 0.8 g/kg soil.

Plants grown in soils treated with molasses developed greater shoot weight (Fig. 2A) and were taller (Fig. 2B) than those grown in soils treated with corresponding treatments without molasses. A small but significant reduction in shoot weight was observed in plants from the treatments with molasses plus the 3 highest urea concentrations; these were the tallest plants in the experiment. Shoot weight reductions in plants from treatments without molasses were negatively related to concentrations of urea in the soil.

Invertase activity was consistently highest in soils treated with molasses and the greatest activity for these treatments corresponded to the 0.4 g rate of urea (Fig. 3A). Treatments resulting in lowest invertase activity were those that contained the 2 highest rates of urea without molasses.

Differences in urease activity in soils treated with the 3 lowest rates of urea irrespective of molasses content were not significant (Fig. 3B). However, the two highest rates of urea resulted in marked increases in the activity proportional to the amount of urea used; for these urea rates, activities in soil with molasses was several-fold that of those without the carbon source.

Ammoniacal N, $\text{NO}_3\text{-N}$ and conductivity were higher in soils from treatments receiving no molasses and urea rates higher than 0.2 g/kg soil than in soils from corresponding treatments with molasses (Fig. 4A-C). Significant positive correlations were established between values for each of the 3 variables and concentrations of urea

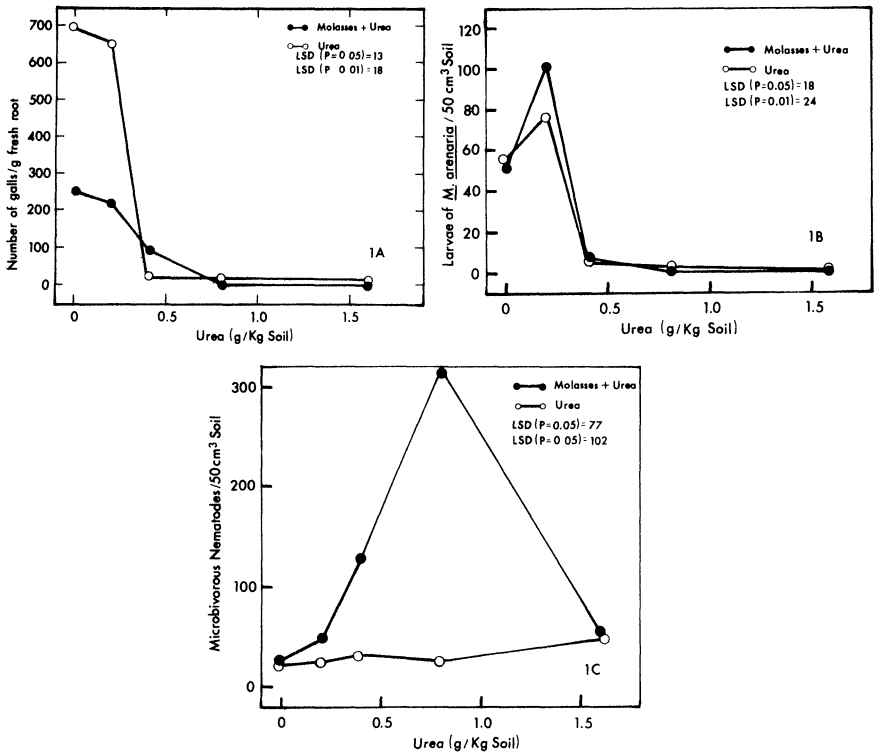


Fig. 1. Effect of urea and blackstrap molasses applied to soil on the number of galls (A) caused by *Meloidogyne arenaria* on roots of squash (*Cucurbita pepo*), larvae of the nematode in soil (B), and soil populations of microbivorous nematodes (C).

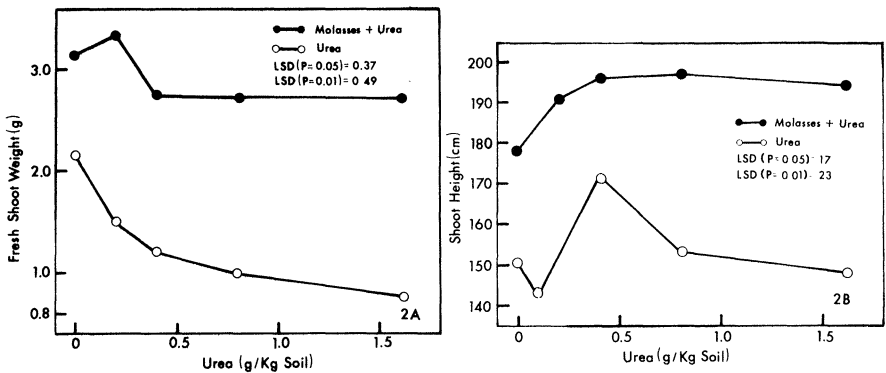


Fig. 2. Effect of urea and blackstrap molasses applied to soil on fresh root weight (A) and shoot height (B) of squash (*Cucurbita pepo*) growing in the soil.

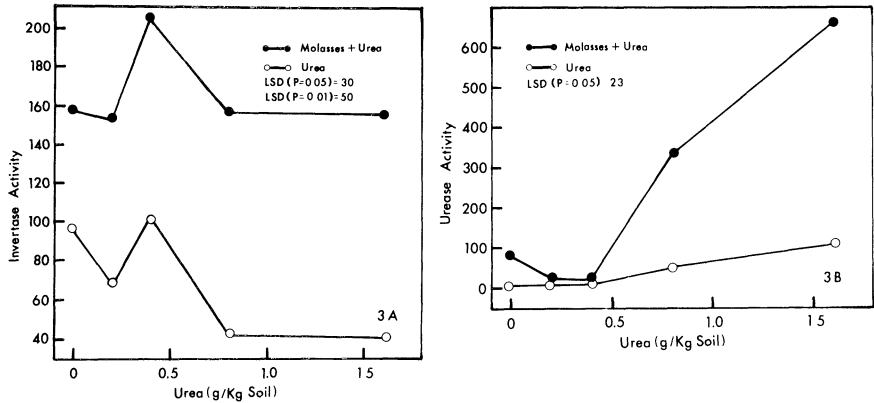


Fig. 3. Relation between concentration of urea applied to soil with and without blackstrap molasses on activities of soil invertase (A) and soil urease (B).

with data from soils without molasses. Similar correlations for molasses-treated soils were not significant.

Soil pH declined sharply in response to the 0.2 and 0.4 g urea rates but did not decline any further in response to higher concentrations of urea (Fig. 4D). Lowest pH values were recorded for soils treated with molasses and the 3 highest urea rates. The highest pH value corresponded to soils that received molasses only.

DISCUSSION

Our results indicate that it is possible to reduce severity of attack by *M. arenaria* with the use of urea alone. The minimal rate required for significant control of the parasite is between 0.2 and 0.4 g/kg soil. The effect of ammoniacal N on nematodes is well documented (4,11,18,20). Applications of anhydrous ammonia or other forms of ammoniacal N repeatedly have been reported to result in control of plant parasitic nematodes. Our results with urea represent a specific case where NH_3 is provided through the action of soil urease on urea resulting in the release of NH_3 and CO_2 . Since the action of urea in soil is dependent on urease, any treatment that results in formation of the enzyme can be expected to increase the efficacy of the chemical against nematodes. The addition of a source of available carbon (molasses) stimulated production of urease activity and significantly improved the efficacy of urea against *M. arenaria*.

The mode of action of the molasses amendment itself involved more than stimulation of urease production. The amendment resulted in significant reduction in severity of galling. Molasses and sugars or other forms of metabolizable carbon added to soil result in increased biological activity by fungi and bacteria, which in turn can result in reduced numbers of plant parasitic nematodes (2,7,9,15). In our case, increased biological activity was quite evident by the significantly higher levels of invertase activity in treatments with molasses compared with those without the C source. A second role played by the molasses amendment in our experiment was that of "detoxification" of the urea treatments. The effective rates of urea against *M. arenaria* were in excess of 448 kg/ha on a broadcast basis. Nitrogen application at those levels caused

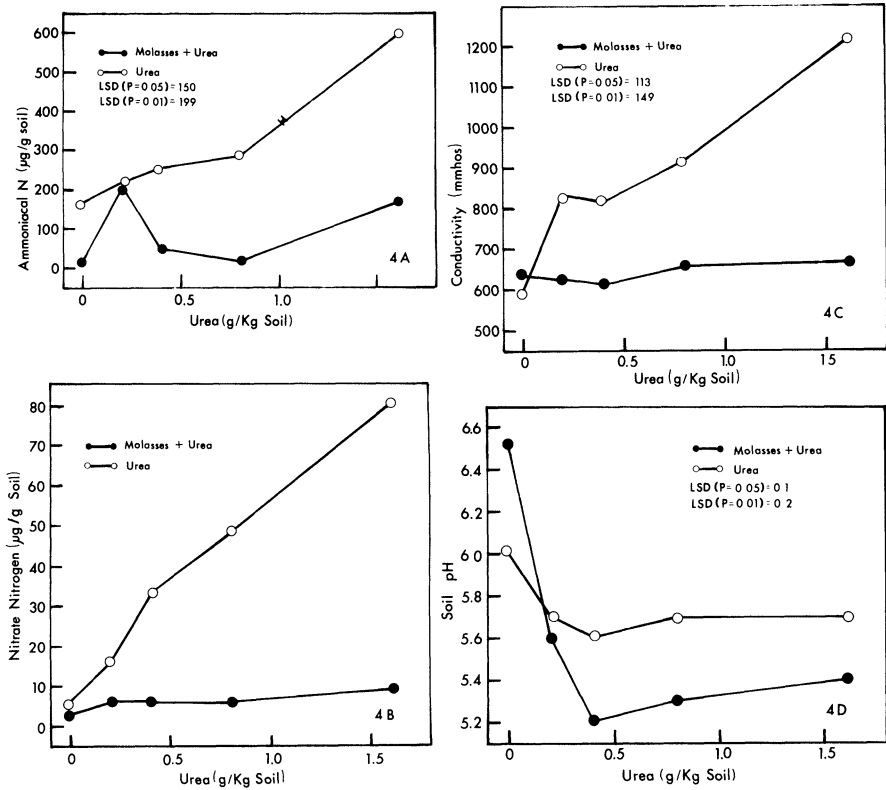


Fig. 4. Relation between concentration of urea applied to soil with and without blackstrap molasses and residual ammoniacal N (A), nitrate N (B), conductivity of a soil water extract (C), and soil pH (D).

an imbalance in the C/N ratio of the soil leading to the observed accumulations of ammoniacal and nitrate N in treatments without molasses. These accumulations were reflected in significantly higher values for conductivity, soil pH, and in marked declines in shoot weights representing significant phytotoxicity from accumulated salts. In contrast, in soils treated with molasses sufficient available C was provided for soil microorganisms to metabolize all the urea-N added so that no significant accumulations of $\text{NO}_3\text{-N}$ or $\text{NH}_4\text{-N}$ were observed.

Increase biological activity in soils with molasses + urea would be expected to result in increased numbers of bacteria and fungi which in turn would explain sharp increases in the number of microbivorous nematodes in some of these treatments. The lack of increases in microbivorous nematodes in response to the highest rate of urea in molasses-treated soil is interpreted as due to a lack of sufficient C to metabolize all the urea in this treatment, leading to a situation unfavorable for development of the nematodes.

Practical application of the treatments described will be limited to situations where a

post-treatment waiting period of 2-3 weeks is possible. The use of these treatments will require thorough mixing in soil of urea with a readily metabolizable carbon source. It is possible to formulate urea with such a carbon source and incorporate the mixture into soil. The cost of the treatments described are relatively high so that at present we consider them useful only in plant nursery beds or in fields with high value crops. The possibility of using nitrification inhibitors to increase the efficacy of the treatments described through reductions in the amount of urea required for nematicidal activity is presently being explored by our group. If successful, the use of these inhibitors could reduce the cost of the treatment significantly.

RESUMEN

La incorporación de urea al suelo en concentraciones de 0.4 g/kg o superiores resultó en reducciones significativas en el número de nódulos por gm de raíz de calabacín (*Cucurbita pepo*) causados por *Meloidogyne arenaria*. Estos tratamientos con urea también resultaron en acumulaciones significativas de nitratos y de nitrógeno amoniacal en el suelo así como en aumentos en la conductividad de los extractos acuosos de suelo y fitotoxicidad pronunciada en las plantas. La incorporación de mezclas de urea con melaza de caña al suelo resultó en un mejor control de *M. arenaria* sin acumulación de nitratos o nitrógeno amoniacal y sin fitotoxicidad en las plantas. Los tratamientos de urea con melaza causaron aumentos en las actividades de la invertasa y la ureasa del suelo y aumentos en el número de nematodos microbívoros en aquellos tratamientos con concentraciones de urea de 0.4 y 0.8 g/kg de suelo.

Claves: fertilidad del suelo, combate de nematodos, nematodos noduladores, proporción C/N.

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