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Mixtures of Olive Pomace with Different Nitrogen Sources for the Control of *Meloidogyne* spp. on Tomato[1]

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Abstract: The efficacy of mixtures of dry olive (*Olea europea*) pomace with biuret, guanidine, and melamine for control of root-knot nematodes (*Meloidogyne* spp.) on tomato (*Lycopersicon esculentum*) was studied in greenhouse experiments. Olive pomace (OP) applied pre-plant at 10 g/kg soil was phytotoxic. Mixtures of OP (10 g/kg soil) with biuret or guanidine at 200–300 mg/kg soil reduced or eliminated the phytotoxic effect, controlled root-knot nematodes, and increased soil esterase activity indicative of microbial activity. The addition of biuret or guanidine without OP to soil at rates <300 mg/kg soil did not control root-knot nematodes. Melamine applied at 100–400 mg/kg soil was phytotoxic as were mixtures of melamine with OP. Treatment of OP with anhydrous ammonia increased N content of the material. In another greenhouse experiment, NH_3-treated OP added to soil was not phytotoxic to tomato, suppressed root-knot nematodes, and increased soil esterase activity. Greenhouse and microplot experiments with OP plus chicken litter demonstrated the efficacy of these combination amendments to control root-knot nematodes and increase tomato yields in *Meloidogyne*-infested soil.

Key words: amendments, anhydrous ammonia, biuret, chicken litter, control, guanidine, melamine, *Meloidogyne* spp., nematode, *Olea europaea,* olive pomace, tomato, root-knot nematode.

Olive (*Olea europaea*) pomace (OP), a waste product from oil extraction processes, is produced in large quantities in Spain, Italy, and other Mediterranean countries. Ecologically appropriate disposal of this material may be a problem because its uses as fodder and as a cattle feed additive are limited (10). Olive pomace is suppressive to root-knot nematodes (*Meloidogyne* spp.) when incorporated in soil (15). It contains propionic and other low-molecular-weight monocarboxylic acids, which are toxic to many plant species (5,11,12). Phytotoxicity of OP amendments to soil is reduced or eliminated when the material is mixed with urea. The OP-urea mixture has a lower C:N ratio, which stimulates microbial degradation of the phytotoxic components of OP and enhances its nematicidal activity (15); however, due to its high water solubility, urea is leached easily from soil. This limits the potential use of OP-urea mixtures as soil amendments to suppress root-knot nematodes in field conditions. It is thus important to find alternative N sources to urea that would persist in soil and detoxify OP while retaining its nematode suppressive properties.

Alternative N sources could include urea polymers or analogs, anhydrous ammonia (NH_3), or chicken litter. Chian (4) showed that urea polymers and analogs such as biuret, guanidine, melamine, and thiourea can be nematotoxic when added to soil at normal N fertilizer rates. These compounds are also toxic to a number of soilborne phytopathogenic fungi (2,3). Anhydrous NH_3, a fungitoxic and nematotoxic compound (13,14), has been used for decades to treat hay and other fodder materials to increase their N content and feed value to cattle. The value of chicken litter as fertilizer and to suppress phytonematodes and other soilborne pathogens has been reported (13,14,17). Chicken litter is also readily available in some locations and inexpensive. Our objective was to assess the value of mixtures of OP with urea analogs and polymers, NH_3, and chicken litter as soil amendments for detoxification

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of OP and for the control of root-knot nematodes.

Materials and Methods

Dry OP was obtained from Compañía Oleícola de Refinación y Envasado (COREYSA), Osuna, Seville, Spain. Chemical composition was similar to that of batches used in previous studies (15). Three organic sources of N (biuret, guanidine, melamine), one inorganic source (NH_3), and chicken litter were studied in combination with dry OP at different rates.

Organic nitrogen compounds: Three separate greenhouse experiments were performed—one each with biuret, guanidine, and melamine as N sources. The soil used in all the experiments was a sandy loam (85% sand, 12% silt, 3% clay; pH 8.1; 1% organic matter; and cation exchange capacity 10 meq/100 g soil) from a cultivated field at the Institut de Recerca i Tecnologia Agroalimentàries (IRTA) Cabrils center. The soil was sieved (5 mm) and steam-pasteurized at 70 C.

Meloidogyne eggs and second-stage juveniles (J2) were obtained from tomato (*Lycopersicon esculentum*) cv. Roma. Whole root systems were macerated in a 0.12–0.15% NaOCl solution (7). Nematodes were collected on a 25-µm-pore sieve (500 mesh).

The soil used in the experiment with melamine was infested with *Meloidogyne arenaria* at 2,100 eggs and J2/kg soil. The species used for the guanidine and biuret experiments was *M. javanica,* adjusted to 1,200 J2/kg soil. Treatments in each experiment were 0, 100, 200, 300, or 400 mg/kg each of the N compounds combined with 0 or 10 g OP/kg soil. The isolate of *M. arenaria* was originally collected from tomato in Cabrera de Mar, Barcelona, and *M. javanica* from fig (*Ficus carica*) in Cabrils, Barcelona.

The treatment procedure was the same for all experiments. Soil was divided into two portions of 40 kg; both were infested with *Meloidogyne* eggs and J2 in 200 ml water, and 400 g of OP was added to one of the portions while the other was left untreated. Each portion was then subdivided in five batches of 8 kg each to which were added the test compounds at appropriate rates. Each batch was mixed in an electrical tumbler-mixer to ensure homogeneous distribution of materials through the soil. The treated soil was distributed in 1.5-liter polyvinylchloride pots, watered, and placed in a greenhouse. Twelve days after treatment, 5-cm-tall tomato seedlings cv. Redondo Liso Ace were transplanted, one per pot. During the period of the experiments, greenhouse temperatures ranged between 20 and 28 C. Plants were watered daily and fertilized once a week with 50 ml/pot full-strength Hoagland's nutrient solution (6). Melamine and biuret experiments were terminated 50 days after transplanting; the guanidine experiment was terminated after 80 days. At the end of each experiment, the following data were collected: number of galls per root system, shoot height, fresh shoot weight, fresh root weight, number of surviving plants, and soil esterase activity. In all three experiments each treatment was replicated eight times in a completely randomized design.

Olive pomace treatment with anhydrous ammonia: Dry OP (200 g) was moistened (40 ml H_2O) and exposed at ambient temperature (23–25 C) to an anhydrous ammonia atmosphere in a hermetically closed vessel. Periods of exposure were 0, 0.5, 2, 4, 7, or 16 hours. The OP from each treatment was stored in a sealed glass bottle until used. Nitrogen content was determined by a Kjeldahl procedure (20).

Soil for this experiment was the same as used in the previous experiments. It was infested with *M. incognita* at a rate of 3,020 eggs and J2/kg soil. This isolate was originally obtained from tomato in Amposta, Tarragona, Spain. The OP was added at 5 g/kg soil and treatments corresponded to the time of exposure of OP to anhydrous ammonia. Materials were mixed with the soil in the same manner as described for the previous experiments. A control treatment without OP was included. Twelve

days after application of OP-NH_3 treatments, 6-cm-tall tomato seedlings Redondo Liso Ace were transplanted, one per pot. Plants were harvested 60 days after transplanting. Data collected were the same as described for the other greenhouse experiments.

Olive pomace and chicken litter: Dry chicken litter was obtained from IRTA Center at Mas Bove, Tarragona, Spain, and ball-milled and sieved to <0.1-mm particle size. The material was partially decomposed and contained 2.59% N according to a Kjeldahl procedure (20). Soil was the same as for the other experiments and was infested with *M. arenaria* at 3,400 eggs and juveniles, as described above. The soil was divided into two 32-kg portions; one portion was left untreated and the other was mixed with OP at 5 g/kg soil. Each portion was then subdivided into four 8-kg aliquots to be mixed with chicken litter. Chicken litter was applied to OP-treated and untreated soil at 0, 2, 4, or 6 g/kg soil. Each treatment was replicated eight times, each replication consisting of 1 kg soil in a 1.5-liter pvc pot. Pots were placed in a greenhouse and kept moist for 2 weeks. The pots were then planted with tomato seedlings as described for the other experiments. The experiment was terminated 60 days after planting. Data collected were the same as for the other experiments in the study.

Microplot experiment: An experiment was established to evaluate the efficacy of a mixture of OP plus chicken litter for control of *M. incognita* in shade-house tomato. Soil for the experiment was sandy (90% sand, 7% silt, 3% clay; pH 7.5; <2% organic matter). The soil was pasteurized as for the other experiments and apportioned into 5-kg amounts in plastic bags. Soil in four bags was infested with *M. incognita* at 2,000 eggs and J2/kg soil. Each bag received an appropriate treatment as described in the following paragraph and, after thorough mixing, the contents of the bags were transferred to 7-liter buckets (1).

Treatments in the experiment were as follows: 1) control without amendment, 2) OP at 10 g/kg soil, 3) chicken litter at 10 g/kg soil, and 4) OP plus chicken litter each at 10 g/kg soil. Each treatment was replicated five times in a completely randomized design. The equivalent of 2 liters gravel was placed at the bottom of each bucket (microplot) before the addition of soil to permit adequate drainage. Buckets were buried in soil to within 5 cm of the rims and spaced 80 cm apart in an open shadehouse in a field, where they were kept for 5 months.

Fifteen days after the treatments were established, each microplot was planted with a 10-cm-tall tomato seedling cv. Redondo Liso Ace. Plants were watered as needed and, after the first month, fertilized every 3 weeks (5 g/microplot of Osmocote Plus (Grace-Sierra Horticultural Products) 16-8-12 (NPK) plus minor elements). Shadehouse temperatures fluctuated between 17 and 32 C.

Data on yield and fruit size were obtained for a 50-day period beginning 80 days after transplanting. At the end of the experiment, galling was assessed according to Zeck's (22) root-knot nematode galling index. Final nematode population densities (roots and soil) were also determined. Nematodes in soil were recovered by centrifugal flotation (9) from a homogenized 250-cm^3 subsample. Nematodes in roots were extracted from whole root systems cut into 2-cm-long pieces followed by maceration in 0.30% NaOCl solution with a blender at 14,500 rpm for 30 seconds in three bursts of 10 seconds each. The resulting nematode suspension was concentrated on nested 150-, 74-, and 25-μm-pore sieves (100, 200, and 500 mesh, respectively). Root tissue and debris were collected on the 150-μm sieve and discarded. Nematode eggs and J2 were collected on the 74- and 25-μm-pore sieves.

Determination of soil esterase activity: Soil esterase activity was measured by incubation of soil directly with an aqueous solution of fluorescein diacetate (FDA). FDA is a non-fluorescent substrate hydrolyzed by various esterase enzymes yielding acetate and fluorescein, a compound with maxi-

mum optical absorbance at 490 nm. The amount of fluorescein released during incubation is directly related to the amount of esterase activity. In this study, we used the method described by Schnurer and Rosswall (16). In each determination, we included soil samples previously autoclaved (no enzymatic activity) and soil without the substrate to account for background color of the soil water extract and non-enzymatic degradation of the substrate. Enzymatic activity was expressed as the increase in optical density at 490 nm of the soil water extract after incubation of the FDA plus soil reaction mixture for 3 hours.

Statistical analyses: All data were subjected to analysis of variance. Fisher's least significant differences (LSD) among means were calculated when F values were significant at the 5% or lower level of probability. Calculation of coefficients of determination and curve fitting were also according to standard procedures for correlation and least-squares analyses. Unless otherwise stated, all differences referred to in the text were significant at the 5% or lower level of probability.

Results

Organic nitrogen compounds: Application of guanidine resulted in plants with heavier shoots than those in untreated soil (Fig. 1A). There were no differences in fresh shoot weight among plants in response to guanidine levels between 100–400 mg. Addition of OP to soil depressed shoot weights; however, mixture of guanidine with OP resulted in increasing shoot weights in response to guanidine rates in the range of 0–300 mg. There were no differences in shoot weight between plants from soils treated with guanidine at the two highest rates with or without OP.

Applications of guanidine alone either had no effect or resulted in a slight increase in weights of fresh roots (Fig. 2A). OP depressed root weights, an effect that was partially offset by addition of guanidine in a pattern of responses similar to that observed for shoot weights.

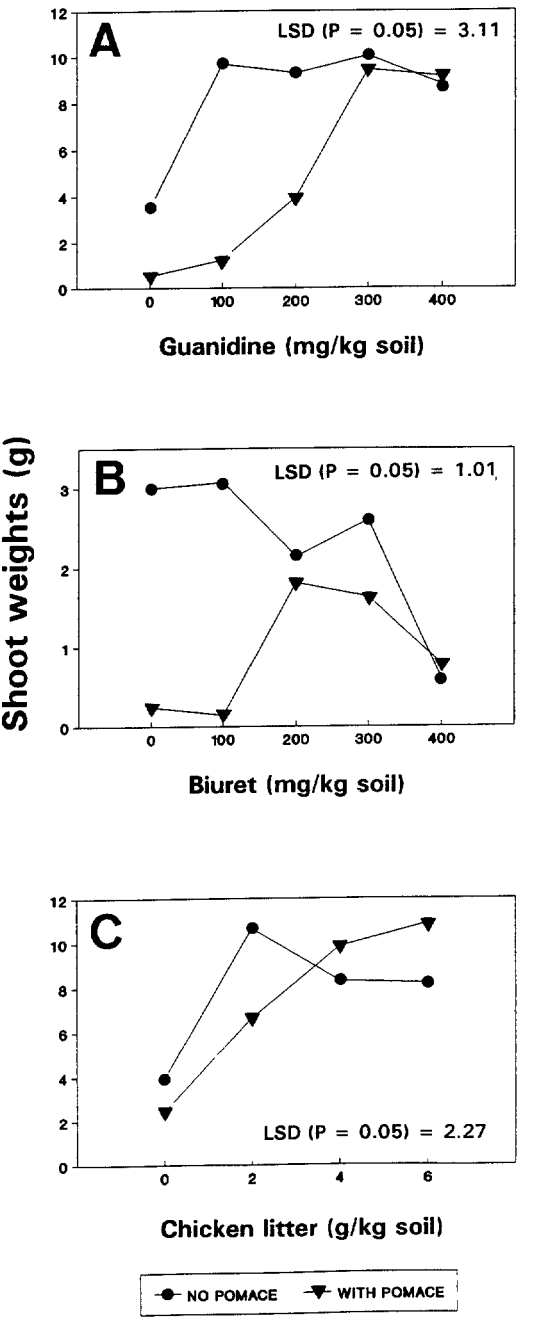

FIG. 1. Effects of amendments with olive pomace with guanidine (A), biuret (B), or chicken litter (C) on fresh shoot weight of tomato cv. Redondo Liso Ace in three greenhouse experiments with soil infested with *Meloidogyne javanica* (guanidine, biuret) or *M. arenaria* (chicken litter). Olive pomace at 10 g/kg soil in A and B, and at 5 g/kg soil in C.

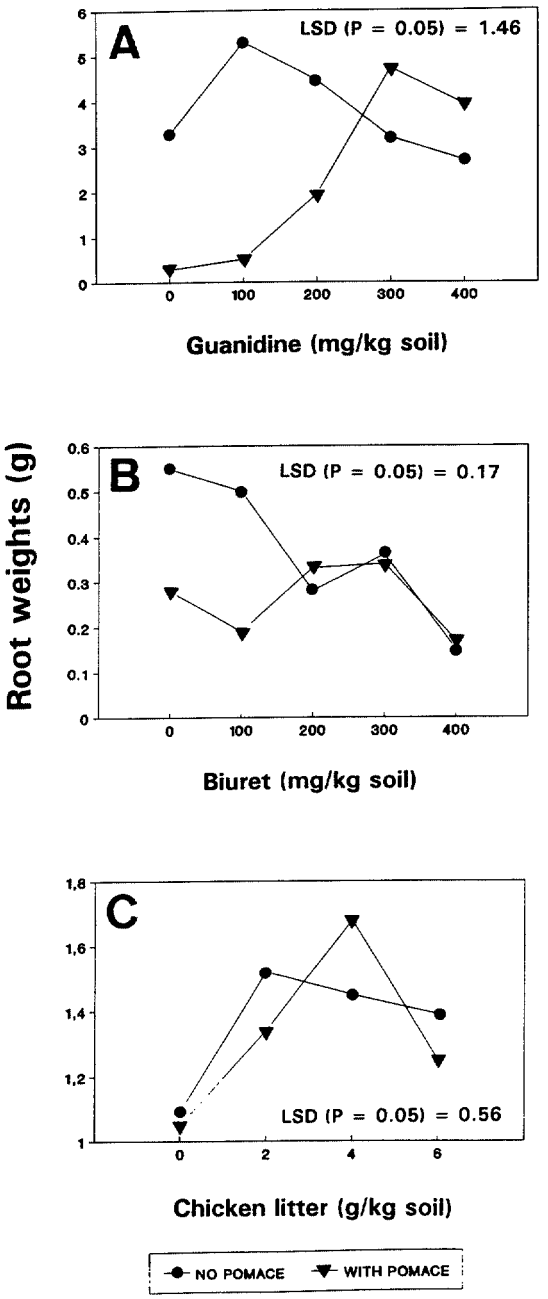

FIG. 2. Effects of amendments with guanidine (A), biuret (B), or chicken litter (C) on fresh root weights of tomato cv. Redondo Liso Ace in three greenhouse experiments with soil infested with *Meloidogyne javanica* (guanidine, biuret) or *M. arenaria* (chicken litter). Olive pomace 10 g/kg soil in A and B, and at 5 g/kg soil in C.

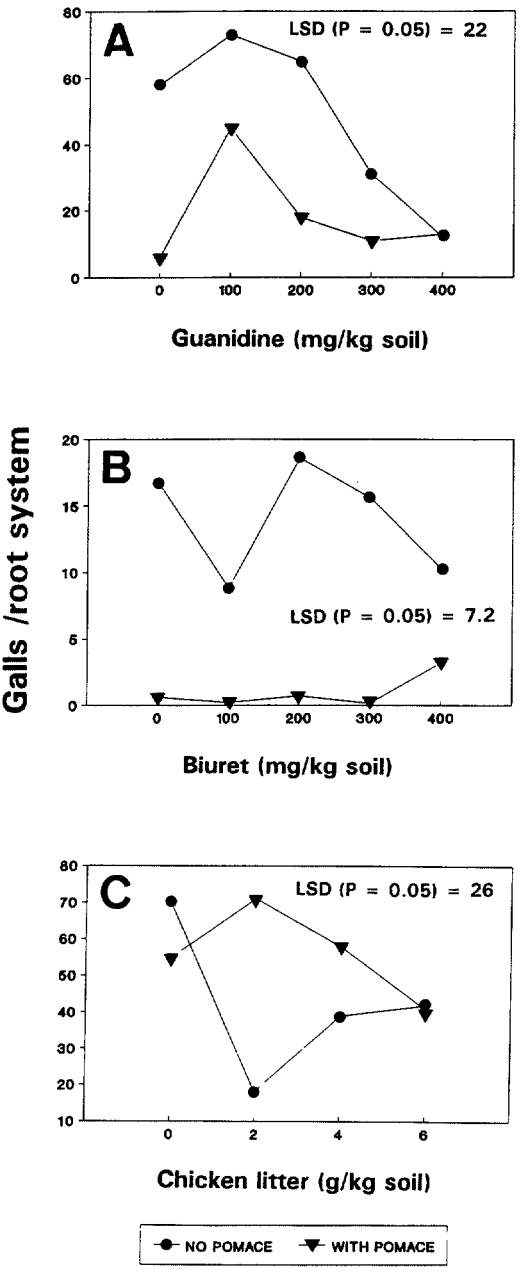

FIG. 3. Effects of amendments with guanidine (A), biuret (B), or chicken litter (C) on number of galls in tomato cv. Redondo Liso Ace roots in three greenhouse experiments with soil infested with *Meloidogyne javanica* (guanidine, biuret) or *M. arenaria* (chicken litter). Olive pomace at 10 g/kg soil in A and B, and at 5 g/kg soil in C.

The addition to soil of guanidine alone at 300- and 400-mg levels reduced numbers of galls per root system (Fig. 3A); lower levels of the compound had no effect on galling. The number of galls per root system was lower in all treatments with OP plus guanidine in the range of 0–300 mg. There was no difference in gall numbers between treatments with 400 mg guanidine.

Esterase activity in soils with OP was twice as high as in soils without it (Fig. 4A). There was no discernible relation between esterase activity and guanidine levels.

Application of biuret without OP resulted in declining weights of fresh shoots (Fig. 1B) and roots (Fig. 2B) in proportion to biuret rates. OP applications reduced shoot and root weights. The mixture of OP with biuret at 200 and 300 mg resulted in heavier shoots and roots than those of other OP treatments.

Biuret alone had no effect on numbers of galls per root system (Fig. 3B). Roots from all OP treatments had few galls.

Esterase activity was 2–3 times higher in soils with OP than in those without it (Fig. 4B). There was no change in esterase activity in response to biuret levels in soils without OP; however, in soils with OP, esterase activity increased in response to biuret levels in the range of 0–200 mg with no additional increase observed for levels >200 mg.

Treatments with melamine were phytotoxic at all levels tested (data not presented). There were no surviving plants at the end of the experiment in pots with melamine concentration >200 mg-kg soil. Esterase activity (data not presented) was higher in soils with OP than in those with melamine alone. In soils with OP, esterase activity increased in response to melamine rates with the highest values occurring in soils with ≥200-mg rates.

Nitrogen content of OP increased with time of exposure to NH_3. The relation between percentage N content and time in hours (T) was described ($R^2 = 0.94$) by the equation $N = 10.66 - 6.71e^{-T}$.

Weights of fresh shoots and roots among plants from pots with OP treatments were lowest for those with non-ammoniated material (0 hours) and highest for plants from soils amended with OP ammoniated for 2–7 hours (Fig. 5A,B).

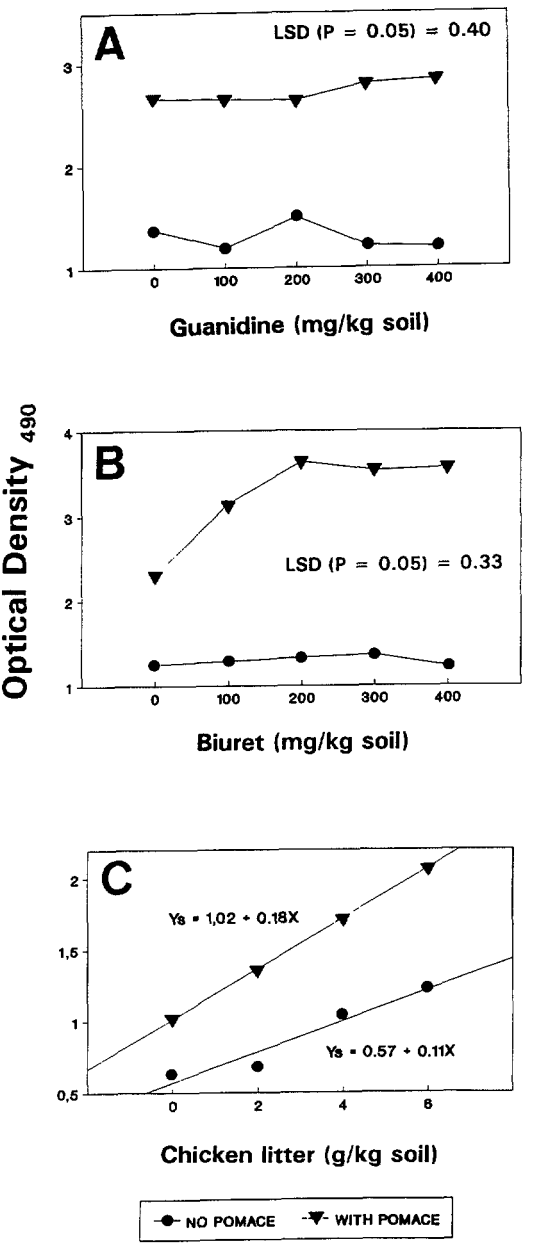

FIG. 4. Effects of amendments with guanidine (A), biuret (B), or chicken litter (C) on soil esterase activity (substrate = fluoresceine diacetate) in OD_{490} units in three greenhouse experiments with soil infested with *Meloidogyne javanica* (guanidine, biuret) or *M. arenaria* (chicken litter). Olive pomace at 10 g/kg soil in A and B, and at 5 g/kg soil in C.

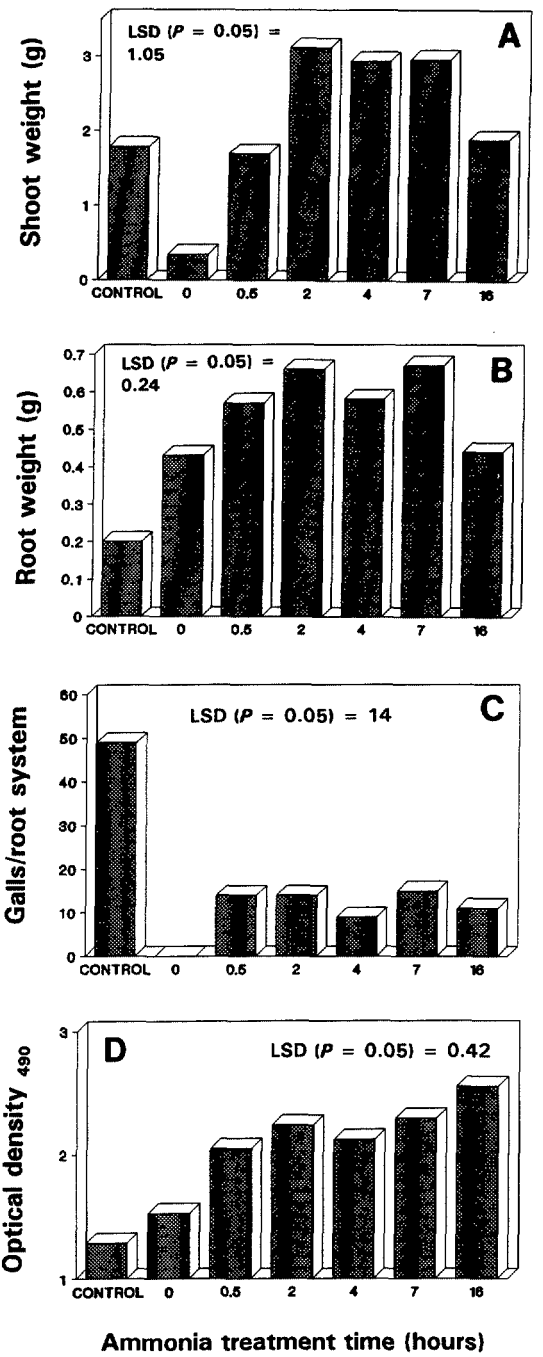

FIG. 5. Relation of amendments with olive pomace exposed to NH_3 atmosphere for various times and growth of tomato cv. Redondo Liso Ace in a greenhouse experiment with soil infested with *Meloidogyne arenaria* (chicken litter). A) fresh shoot weight. B) fresh root weight. C) number of root galls induced by the nematode. D) soil esterase activity (substrate = fluoresceine diacetate) in OD_{490} units.

All OP treatments resulted in lower numbers of galls per root system than the untreated control (Fig. 5C). There was no relation between ammoniation time and the number of galls in plants growing in OP-treated soil.

Esterase activity (Ys) of soils treated with OP (Fig. 5D) increased in relation to ammoniation time (R^2 = 0.80) according to the equation $Ys = 1.75 + 0.221T^{1/2}$. The relationship between esterase activity and percentage N in OP was $YS = 2.59 - 7.24e^{-N}$ (R^2 = 0.83).

Application of chicken litter alone resulted in increased weights of fresh shoots and roots (Fig. 1C,2C); this response was attained with the 2-g rate, and no additional increase was obtained with higher levels of litter. OP alone did not affect weights of roots or shoots. Amendments with chicken litter plus OP resulted in increased weights of shoots and roots; this response was proportionate to the amount of chicken litter added in the range of 0–4 g. Application of OP plus 6 g chicken litter had no effect on shoot weight but reduced root weight sharply below that corresponding to the OP plus 4 g chicken litter treatment.

All treatments with chicken litter alone suppressed numbers of galls per root system compared with the untreated control (Fig. 3C); the only OP plus chicken litter treatment suppressive of gall formation was that with the 6-g rate of litter.

Soil esterase activity increased linearly in response to the amount of litter added (Fig. 4C). Soil with OP treatments had 1.6 times the activity of those without OP.

Microplot experiment: Tomato yield was highest in plots with chicken litter, OP, and the OP plus chicken litter treatments (Table 1). There were no differences in average fruit diameter in response to the various amendments, nor between the control and the other treatments in the experiment. The highest root-knot nematode galling indices were in control plants and were lowest in plants with chicken litter followed in ascending order by those with OP plus chicken litter and with OP alone.

TABLE 1. Effect of olive pomace (OP) alone and in combination with chicken litter on yield and control of *Meloidogyne incognita* 130 days after inoculation with 2,000 nematodes/kg of soil in a shadehouse microplot experiment.

Treatment	Yield kg per plant	Fruit diameter (cm)	Galling index[a]	Final population (roots and soil) 1,000s	Nematodes per g of root 1,000s
Control	1.48	6.3	9.6	4,397	42.4
Chicken litter	2.28	6.3	0.2	2	0.1
OP + Chicken litter	2.13	6.0	2.5	385	9.4
OP	2.20	6.0	4.1	1,546	25.7
LSD ($P \leq 0.01$)	0.28	NS	1.6	3,442	23.3
LSD ($P \leq 0.05$)	0.21	NS	1.2	2,524	17.1

All numbers are means of five replications.
[a] Galling index based on a scale of 0 = no galls . . . 10 = 100% of root system galled (22).

The lowest numbers of nematodes in the roots and the lowest total final population densities (roots plus soil) were observed in plants and plots with the chicken litter, chicken litter plus OP, and OP treatments; there were no differences in nematode numbers among these treatments.

DISCUSSION

Results obtained in greenhouse and microplot experiments confirm findings on the nematicidal properties of OP (15). Phytotoxicity of this material was reduced or eliminated by ammoniation with NH_3 and by combinations with guanidine, biuret, and chicken litter. Soil esterase activity determined with FDA as substrate correlates well with microbial activity in soils (23), soil and litter (16), container media (8), and in other environments (18). The narrowing of the C:N ratio of the amendments by addition of these nitrogenous compounds increased microbial activity as evidenced by increased esterase activity. Increased microbial activity is one of the characteristics of organic amendments to soil suppressive of phytonematodes (13,14,17) and other soilborne pathogens (8).

Guanidine may be thought of as an analog of urea and, like urea, is soluble in water but contains considerably more N (71.14%) than urea (46.65%). On heating at 160 C, guanidine is converted to melamine (66.6% N), which is slightly soluble in water. Biuret is a dimer of urea formed also by heating this compound; it contains 40.8% N but is considerably less soluble in water (2.01% (w/w) at 25 C) than urea. Our experiments with these compounds thus provided NH_2-N at a range of water solubilities and in chemical forms that could have influenced the availability of N through different rates of decomposition by microbial activities. Our results with guanidine and biuret indicate that N in these experiments was available for detoxification of OP. The data suggest also that it may be advantageous to combine OP with these compounds to provide the nitrogen necessary for stimulation of microbial activity and detoxification of OP.

Melamine is a triazine, several of which are commercial herbicides used to control primarily dicotyledoneous weeds (21). Melamine was phytotoxic to tomato in our study. Nevertheless, esterase activity in soils with OP plus melamine was much higher than that of soils with OP or melamine alone, indicating that melamine N was available to microorganisms. It is possible that melamine plus OP combinations may be useful where triazine-resistant crops are grown. It is also possible that melamine at rates lower than those in our experiment may be less toxic to tomato and useful for combinations with OP.

Of the three organic N compounds tested, guanidine was the least phytotoxic and most compatible with OP for tomato production. In soils with OP treatments, shoot and root weights increased directly in relation to the amount of guanidine

added. This was also true for biuret; however, amendments with biuret alone resulted in significant reductions in shoot and root weights at rates >200 mg/kg soil, indicating phytotoxicity from the compound.

Treatment of OP with NH_3 resulted in Maillard-type reactions. The material became blackened even after only 30 minute exposure to the gas. OP contains a great deal of xylan and other hemicelluloses (11) that react with NH_3 and amines forming Maillard and alkaline condensation compounds (19). As a result of these reactions, amine bonds are formed so that a portion of the NH_3-N is effectively bound to OP. Results showed that N content of OP could be increased with simple room temperature exposure to NH_3. Ammoniated OP was not phytotoxic, stimulated microbial activity (increased esterase levels), and was suppressive to root-knot nematode. Treatment of OP with NH_3 would be simple to develop for large-scale applications.

Mixtures of chicken litter with OP were not phytotoxic. These combination treatments were somewhat suppressive to root-knot nematodes. Because chicken litter was the least expensive and most readily available of all N sources, we chose this material for the microplot field study. The microplot experiment represented a long-term, full-season situation under similar conditions as those for shadehouse tomato production in Spain. Data from OP plus chicken litter amendments confirmed results obtained in the greenhouse. Nematode control was obtained with the mixture without phytotoxicity and with significant (44%) yield increase over the control. The addition of OP to chicken litter did not improve yield over what was obtained with chicken litter alone. However, as demonstrated by the greenhouse experiment, the OP plus chicken litter combination increased microbial activity over what was obtained with the OP amendment alone. This may explain the improved nematode control obtained in the microplot experiment with the OP plus chicken litter treatment over what was obtained with OP alone. In other work, we observed weed and Fusarium wilt control in tomato in soils with OP treatments but not in those with chicken litter alone (unpubl.). In summary, results from this study demonstrated several practical possibilities for formulating OP that permit integration of this waste product into horticultural operations typical of Spain and other parts of the Mediterranean region, where OP residues are a problem.

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