Organic matter (OM)–based amendments offered to farmers vary greatly in their source, properties, and effectiveness. Some of these are produced from natural organic resources, whereas others originate from recycled organic waste, in part as an environment-friendly low-cost alternative to waste disposal. Stimulatory effects of OM on plant growth have been noticed in many systems, but a few reports have indicated negative effects. Many factors and interactions dictate plant response to these materials, and not all are well understood. Procedures that can be used to evaluate OM composition and characteristics that might affect plant growth are complicated and expensive. Deleterious effects of OM additions are frequently associated with a nonmature status of the decomposing material, but maturity is difficult to define. Evaluation of humic substances (HS)–based products is even more complex and needs improvement of the available methodologies. This article presents two simple versatile bioassay methods carried out in microplates or pouches for screening and evaluating soluble organic products for agronomic and horticultural use. To illustrate the two methods, we present microplate experiments with creeping bentgrass (Agrostis palustris Huds.) that provide an evaluation of plant response to N rates spanning from deficient to excessive levels (0.9–57.6 mg N L⁻¹) and to 11 different soluble HS products. The pouch method is presented by soybean (Glycine max L.) and corn (Zea mays L.) experiments that evaluate plant response to two HS and determine whether it is related to Fe or Zn mediation by the tested substances. The two methods are shown to be sensitive for nutritional factors and could effectively rank various OM materials from being deleterious to stimulatory to plant growth. (Soil Science 2008;173:342–349)

Keywords: Bio-assay, humic substances, organic matter, plant response.
continued from that time up to the present by farmers all over the world. Yet, some studies are known where negative effects were caused by OM application. The beneficial effects, once believed to result from direct OM nutrition of plants (the "Humus Theory"), are known today to be related to many indirect and not always well-defined processes. The effects on mineral nutrition of plants, by supplying nitrogen (N), phosphorus (P), potassium (K), and/or other macronutrients and micronutrients released by microbial mineralization of the OM, are well known. Other indirect effects are found, such as by increasing soil cation exchange capacity or by promoting reestablishment of beneficial microbial populations in disturbed soils. Specific effects by means of mediation and mobilization of micronutrients from mineral components of the soil have been reported and thoroughly reviewed (Chen and Aviad, 1990; Chen, 1996; Chen et al., 2000; Chen et al., 2004a,b; Chen and De Nobili, 2004).

Improving soil structure, aeration, and water retention by OM are known as well to be beneficial for plants (Clapp et al., 2001). Detoxification of phytotoxic constituents, such as boron (B) or aluminum (Al) when they are present in high concentrations in the soil or in the irrigation water, is another mechanism that was shown to promote plant growth (Yernuyahu et al., 2001). Some studies suggest a direct effect on membrane permeability or hormone-like effect of discrete, mostly unknown compounds of the soluble OM on plant growth (Nardi et al., 2000; Pizzeghello et al., 2001). The deleterious effects are usually associated with low degradation of the OM, referred to as immaturity when compost is used (Zmora-Nahum et al., 2005). They may result from competition for oxygen (O_2) and/or N (mostly as NO_3^- or NH_4^+) because of the biologically unstable nature of such materials or to the high ratio of carbon (C) to N. They also may result from the presence of allelopathic compounds released by the decomposed plants or from secondary microbial metabolites and degradation products, as well as other specific phytotoxic organic substances, such as propanoic acid and n-butyric acid, or from high salinity (Chanyasak et al., 1983; Hadar et al., 1985; Blanco and Almendros, 1997; Wu and Ma, 2001). Requirements for stability or maturity are considered essential, but no widely accepted criteria, testing procedures, or standards are defined for evaluation of organic amendment quality (Senesi et al., 1996). Recently, the dissolved organic carbon content in compost extracts has been proposed as a universal criterion for compost maturity (Zmora-Nahum et al., 2005).

A variety of organic amendments have been used as fertilizer supplements in crop production and in horticultural uses, especially in turfgrass management. They not only enhance fertilizer efficiency for plant growth, but also reduce the potential for groundwater contamination (Hunter and Anders, 2004). Common examples are humic substances (HS), seaweed products, waste materials, manures, biosolids, composts, and peat moss. Among them, HS are the most commonly used materials in crop and turfgrass management. Humic substances consist of humic acids (HA), fulvic acids (FA), and humin. Owing to the insoluble nature of humin, it is not used as a soil amendment. The positive effect of HA on turfgrass growth in golf courses is a common observation, but only a few journal articles have described these effects and experimentally defined the mechanisms involved (e.g., Chen et al., 2004a; Zhang and Ervin, 2004).

Because plant response to organic amendments involves a series of mechanisms, not always defined for a given material, and because evaluation methods, criteria, and standards are not always conclusive, a screening method that would allow evaluation of the vast variety of organic amendments offered for agronomic use is needed. The objective of this research was to present a rapid method for screening various organic materials using newly developed laboratory screening techniques for examining growth enhancement of turfgrass and agricultural crops by HS. A "microplate" method, adapted from Nelson and Craft (1992), and a "pouch" method were designed for this purpose. These methods were evaluated by testing plant response to N fertilization rates and were used to compare laboratory-prepared and commercially produced HS products with fertilizer controls for plant growth parameters. Selected results from these experiments and other previously published studies (Chen et al., 2004a) provide essential information for field experiments or for practical use of HS on golf courses and sports turf.

MATERIALS AND METHODS

**Humic Substances**

Laboratory-grade materials included HA from fibric, hemic, and sapric peats (from Minnesota) in
addition to HS extracted (by the IHSS method; Swift, 1996) from Irish and British organic soils and plant material as follows: Norfolk peat (Norfolk Fens, England; a basin peat) HA and FA, Kerry (Ireland) highland peat HA and FA, and the HA and FA isolates from moss. Commercially produced HS samples (Horizon Ag Inc.) were Leonardite HA and a Leonardite HA-FA mix. Bulk HS concentration in our plant growth experiments was 10 mg L⁻¹.

**Fertilizers**

In the experiments conducted to investigate the effects of the HS materials, a nutrient solution (NS) of 3.6 mg L⁻¹ N and K and 0.9 mg L⁻¹ P was prepared from solutions of NH₄NO₃ (6.6 mg L⁻¹), KNO₃ (9.3 mg L⁻¹), and Ca(H₂PO₄)₂ (3.4 mg L⁻¹). In experiments used to investigate the effects of N rates on plant growth, N concentrations supplied as NH₄NO₃, varied from 0.9 to 57.6 mg L⁻¹.

**Plants**

The grass species used in the microplate experiments was creeping bentgrass (*Agrostis palustris* Huds., cv. Providence). Crop species used in the pouch experiments were soybean (*Glycine max* L. Merr. cv. Pioneer 91B64) and corn (*Zea mays* L., cv. Dekalb 493).

**Microplates**

Falcon microplates (Fisher Scientific, Pittsburgh, PA) with 12 wells, about 5 mL each, were used (Fig. 1). Four holes (3 mm) were drilled at the bottom of each well. One of the holes held a filter paper wick extending about 5 mm above and below the bottom of the hole to supply the NS. Experiments showed that four holes were optimal for solution exchange and root growth. Fine sand (<1 mm) and coarse sand (1–2 mm) were layered into the well to provide a desired level of water-holding capacity. Six grass seeds were placed in the sand and kept moist with a bottom microplate containing water. After 1 week of germination and growth in a 20°C constant temperature chamber (model GCW15; Environmental Growth Chambers, Chagrin Falls, OH) with a day-night cycle of 16:8 h, three microplates were placed in a plastic tray containing fertilizer only or fertilizer plus HS solutions. The microplates were raised about 8 cm from the tray by small plastic posts, so that the wicks, but not the microplates, were exposed in the solution. Each treatment tray contained a total of 36 wells. Seedlings were then thinned to three plants per well. A “constant-head” bottle was used to provide additional nutrient solution to the plastic tray. Plants remained in the plates for 21 additional days before harvesting.

For harvest, the three plants from each well were taken out and washed with water to remove the sand. After separating shoots from roots, shoots from five wells were combined into one sample; six replicates were collected from each of the treatments. For root dry-weight measurements, roots from two wells were combined into one sample; nine replicates were collected per treatment. Root and shoot samples were placed into vials, dried at 65 °C, then weighed for tissue dry mass. Root length was measured by the method described by Dowdy et al. (1998). Fresh roots were combined from two wells, washed, and placed into 10% methanol solution. The roots were stained overnight with basic fuschin (0.025%) before image processing by scanning on a digitizing video camera and using a Word Image Processing System to evaluate the root length. Triplicate samples were measured for each treatment.

**Pouches**

For the effect of HS materials on larger plants, the microplate wells are too small. A simple setup of 16 × 18-cm nutrient culture growth pouches

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**Fig. 1.** Schematic presentation of the microplate growth system. A, View of the upper microplate with four holes in each well. B, View of the bottom microplate, containing water or nutrient solution. C, View of a single well of the upper microplate, filled with sand. The four holes are shown, one of them holding a filter paper wick.
Fig. 2. A, Pouches in racks with corn plants at harvest stage. B, Pouches with corn plants showing root growth.

Mega International, Minneapolis, MN), which do not need an aeration system, was used to test HS effects on soybean and corn growth (Fig. 2). Five to six seeds were placed in the paper trough of each pouch, which contained 250 mL of the standard NS: Ca(NO\textsubscript{3})\textsubscript{2} \cdot 4H\textsubscript{2}O, 2 mM; K\textsubscript{2}SO\textsubscript{4}, 0.7 mM; MgSO\textsubscript{4} \cdot 7H\textsubscript{2}O, 0.5 mM; KH\textsubscript{2}PO\textsubscript{4}, 0.2 mM; KCl, 0.1 mM; H\textsubscript{2}BO\textsubscript{3}, 10 μM; MnCl\textsubscript{2} \cdot 4H\textsubscript{2}O, 1 μM; CuCl\textsubscript{2} \cdot 2H\textsubscript{2}O, 0.5 μM; and (NH\textsubscript{4})\textsubscript{6}Mo\textsubscript{7}O\textsubscript{24} \cdot 4H\textsubscript{2}O, 0.05 μM. To assess whether plant response to the HS is related to Fe and/or Zn mobilization by the tested HS, no Fe and Zn were included in the NS solution, and the following treatments were applied: (A) control, (NS); (B) NS + FA (10 mg L\textsuperscript{-1}); (C) NS + HA (10 mg L\textsuperscript{-1}); (D) NS + 50 μM FeSO\textsubscript{4} + 5 μM ZnSO\textsubscript{4}; (E) NS + 50 μM Fe-EDTA + 5 μM Zn-EDTA; (F) NS + FA (10 mg L\textsuperscript{-1}) + 50 μM FeSO\textsubscript{4} + 5 μM ZnSO\textsubscript{4}; and (G) NS + HA (10 mg L\textsuperscript{-1}) + 50 μM FeSO\textsubscript{4} + 5 μM ZnSO\textsubscript{4}. To buffer the pH at about 7.3, 2 g analytical CaCO\textsubscript{3} was added to each pouch. Five days after germination, the plants were thinned to three in each pouch and grown for additional 3 weeks in the growth chambers described previously. Five replicate pouches were tested for each treatment. Replacement NS were added to the growth pouches each week. Chlorophyll concentration in the leaves was measured at harvest using a Spad-502 chlorophyll meter (Minolta Corp., Ramsey, NJ). After photography, all plants were removed from each pouch; shoots and roots were separated, dried at 65°C, and weighed.

Data Analysis

The statistical analyses were carried out using a JMP IN software (version 5.0; SAS Institute, Cary, NC). For multiple-range analyses, the Tukey honestly significant difference (HSD) test was used with a P value of 0.05 as a threshold for significance.
RESULTS AND DISCUSSION

**Microplates**

Bentgrass plant response to N rates showed a steady increase for both root and shoot dry weights with increasing N rates up to 10.8 mg L\(^{-1}\) (Fig. 3). Differences in tissue weight of plants grown at N rates between 10.8 and 28.8 mg L\(^{-1}\) were small. When the N rate was as high as 57.6 mg L\(^{-1}\), both root and shoot dry weight significantly decreased. This is consistent with the root length measurement (Table 1) of 93 cm per plant at 57.6 mg L\(^{-1}\) N, compared with 102 cm per plant at an N rate of 28.8 mg L\(^{-1}\).

Higher N rates are known to favor shoot growth. This was demonstrated in the present experiment by a notable decrease of the root-shoot ratio with increasing N rates between 0.9 and 7.2 mg L\(^{-1}\). Although both root and shoot weight in this range increased with increased N levels, the root-shoot ratios decreased. Almost constant root-shoot ratios (between 0.7 and 0.8) were observed at the higher N rates between 7.2 and 57.6 mg L\(^{-1}\). The N content in roots and shoots of the plants increased with increased N concentration in the nutrient solution, but the increase in the shoot was larger, indicating that plant shoots consumed more easily available nutrients than did the roots.

Plant responses to various HS products including HA and FA from laboratory-grade and commercially produced sources are summarized in Table 2. Both root and shoot dry weights increased for some treatments but decreased for others. The root dry weight increased up to 45% (fibric peat HA) over the control, and the shoot dry weight increased up to 69%, with the same treatment (fibric peat HA) exhibiting the highest increase; hemnic peat HA and Leonardite HA also exhibited significant root-weight enhancement compared with the control. Increases of shoot weight were exhibited by the fibric peat HA, Norfolk peat FA, hemic peat HA, and the Leonardite HA treatments. In contrast, the Moss HA, Moss FA, and Kerry soil HA induced a decrease in root mass. These three treatments and the Norfolk peat HA induced also a decrease of shoot dry weight. Plants grown in NS containing hemic peat HA, sapic peat HA, and the Leonardite HA exhibited enhanced root-shoot ratios compared with the control.

**TABLE 1**

<table>
<thead>
<tr>
<th>N rate, mg L(^{-1})</th>
<th>N content, %</th>
<th>Root/shoot ratio</th>
<th>Root length, cm plant(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9</td>
<td>0.96</td>
<td>1.30</td>
<td>1.56(\text{c})</td>
</tr>
<tr>
<td>1.8</td>
<td>0.98</td>
<td>1.59</td>
<td>1.32(\text{a})</td>
</tr>
<tr>
<td>3.6</td>
<td>1.07</td>
<td>2.07</td>
<td>0.98(\text{b})</td>
</tr>
<tr>
<td>7.2</td>
<td>1.45</td>
<td>3.07</td>
<td>0.82(\text{b})</td>
</tr>
<tr>
<td>10.8</td>
<td>1.64</td>
<td>3.11</td>
<td>0.82(\text{b})</td>
</tr>
<tr>
<td>14.4</td>
<td>2.10</td>
<td>3.73</td>
<td>0.87(\text{b})</td>
</tr>
<tr>
<td>28.8</td>
<td>2.91</td>
<td>4.63</td>
<td>0.71(\text{b})</td>
</tr>
<tr>
<td>57.6</td>
<td>3.79</td>
<td>6.16</td>
<td>0.80(\text{d})</td>
</tr>
</tbody>
</table>

Values are means of six replicate wells, three plants per well. Values having the same superscript letter do not differ from each other at \(P < 0.05\) (multiple-range analysis, Tukey HSD).

**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>Dry weight, mg plant(^{-1})</th>
<th>Root-shoot ratio</th>
<th>Root length, cm plant(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>Control</td>
<td>3.80(\text{d})</td>
<td>2.45(\text{d})</td>
<td>1.6</td>
</tr>
<tr>
<td>Hemic peat HA</td>
<td>5.00(\text{a})</td>
<td>2.70(\text{bc})</td>
<td>1.8</td>
</tr>
<tr>
<td>Sapric peat HA</td>
<td>4.39(\text{bc})</td>
<td>2.36(\text{cde})</td>
<td>1.9</td>
</tr>
<tr>
<td>Fibric peat HA</td>
<td>5.52(\text{a})</td>
<td>4.02(\text{a})</td>
<td>1.4</td>
</tr>
<tr>
<td>Norfolk peat HA</td>
<td>3.24(\text{def})</td>
<td>2.03(\text{e})</td>
<td>1.6</td>
</tr>
<tr>
<td>Norfolk peat FA</td>
<td>4.05(\text{bde})</td>
<td>2.91(\text{b})</td>
<td>1.6</td>
</tr>
<tr>
<td>Kerry soil HA</td>
<td>3.33(\text{def})</td>
<td>2.24(\text{de})</td>
<td>1.6</td>
</tr>
<tr>
<td>Kerry soil FA</td>
<td>3.62(\text{cde})</td>
<td>2.33(\text{cde})</td>
<td>1.6</td>
</tr>
<tr>
<td>Moss HA</td>
<td>2.51(\text{f})</td>
<td>1.73(\text{f})</td>
<td>1.5</td>
</tr>
<tr>
<td>Moss FA</td>
<td>2.58(\text{f})</td>
<td>1.96(\text{ef})</td>
<td>1.4</td>
</tr>
<tr>
<td>Leonardite HA</td>
<td>4.47(\text{abc})</td>
<td>2.73(\text{bc})</td>
<td>1.7</td>
</tr>
<tr>
<td>Leonardite FA-HA</td>
<td>4.31(\text{bcd})</td>
<td>2.52(\text{bde})</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Effects were measured by dry weight, root-shoot ratios, and root length using the microplate method. All treatments include NS with N and K at a rate of 3.6 mg L\(^{-1}\) and P at a rate of 0.9 mg L\(^{-1}\). HA and FA rate is 10 mg L\(^{-1}\). Values are means of nine (root) or six (shoot) replicates; each consists of two (root) or five (shoot) wells, three plants per well. Values within a column having the same superscript letter do not differ from each other at \(P < 0.05\) (multiple-range analysis, Tukey HSD).

†N rates are given in mg N L\(^{-1}\), supplied as NH\(_4\)NO\(_3\).

N rates are given in mg N L\(^{-1}\), supplied as NH\(_4\)NO\(_3\).
PLANT GROWTH AND ORGANIC AMENDMENTS

All HS tested in this experiment exhibited positive effects on root length. This result corresponds with the results of the study of Nardi et al. (2000) who found strong evidence that HS stimulate root cell elongation and proliferation. This was demonstrated by scanning electron micrographs that showed abundance of root hairs in wheat in HS-treated roots and also by transmission electron microscopy, which showed that HS induce a higher differentiation rate. This information may be of particular significance to golf course managers. Most commonly, bentgrass is grown in 80% to 100% sand. Application of the appropriate amount of HS may increase the tolerance of the plants to environmental stress due to enhanced root growth.

Overall, 2 of the 11 HS tested in this experiment resulted in enhanced biomass accumulation of creeping bentgrass plants grown in NS-containing fertilizers and supplemented with HS. The application of three additional HS products resulted in growth decrease, and the rest of the tested materials did not significantly affect plant growth. This provides encouraging information that supports application of selected HS on turfgrasses in addition to chemical fertilization, but the high diversity of the effects of HS on plant growth highlights the necessity of quality assurance of the available materials.

Pouches

Plant growth data for pouch-grown soybean plants are shown in Figure 4. Shoot and root dry weights increased (15%-55%) with inclusion of commercially produced HS treatments (either HA or FA) in the NS, compared with the control plants that were grown on NS solution alone. These two treatments resulted also in a slight remedy of plant chlorosis: SPAD reading increased from 24 ± 0.9 (mean ± SEM) for the highly chlorotic control plants to SPAD readings of 32.5 ± 1.2 for the NS + HA—treated plants and 30.1 ± 1.7 for the NS + FA—treated plants. Inclusion of Fe and Zn as sulfate salts in the NS resulted in a remedy of the chlorosis (SPAD reading of 39.6 ± 1.8) and much larger plants, with root and shoot dry-weight increases of 76% and 88%, respectively, over the control. Adding Fe and Zn as complexes of EDTA, FA, or HA resulted in comparable or slightly higher chlorophyll content, as indicated by SPAD readings of 41.9 ± 1.4, 40.5 ± 1.3, and 43.4 ± 1.9, respectively. Shoot and root dry weights all doubled over the control. These observations are in accordance with the enhancement of Fe and Zn uptake by plants treated with HS (the “FeHS theory”; Chen et al., 2004b).

The effects of the same treatments on pouch-grown corn plants are shown in Figure 5. In contrast to soybean plants, root
and shoot dry weight obtained for the corn plants grown in the control solution (NS) was about the same as for the NS + HA or the NS + FA treatments. The plants of these three treatments were slightly chlorotic with SPAD readings of 31.6 ± 1.4, 30.4 ± 0.9, and 29.5 ± 1.2, respectively. Adding Fe and Zn as sulfate salts did not result in an increase of root dry weight and only slightly increased shoot dry weight (44% above the control). Adding Fe and Zn as complexes of EDTA, FA, or HA resulted in much larger shoot and root dry weights (increase of root dry weight by 79%–96% and increase of shoot dry weights by 192%–225% over the control). The positive growth response of the three treatments which included complexes of Fe and Zn was not accompanied by chlorosis remedy. On the contrary, all treatments that included Fe and Zn resulted in the most chlorotic plants with SPAD readings of 26.0 ± 1.5 for the Fe and Zn sulfate treatment, 25.4 ± 0.7 for the EDTA-complexed metals, 23.3 ± 0.5 for the FA-complexed metals, and 23.4 ± 0.7 for the HA-complexed metals. Plant tissues were not analyzed, but severe purple coloring of plants of these four treatments, starting from the old leaves (visual estimation), may suggest P-related disorder that was induced by an extreme enhancement of the availability of Fe and Zn. A different, yet unknown mechanism, could also be involved in inducing these observations.

CONCLUSIONS

Lee and Bartlett (1976) indicated that a positive growth response can be observed only when the application of HA is directed to a soil low in OM or to nutrient solutions. This was further stressed by Chen and Aviad (1990), Chen (1996), and Chen and De Nobili (2004), who linked this effect to mobilization of micronutrients. Our results agree with this statement. Under conditions that induced Fe and Zn deficiencies (CaCO₃-buffered high pH), both HA and FA enhanced plant growth of both soybean (Fig. 4) and corn (Fig. 5). When the N levels were set at 3.6 mg L⁻¹, which was shown to be suboptimal and to induce N stress in creeping bentgrass (Table 1), positive responses were obtained for some of the HS but not of others (Table 2). The physiological mechanisms involved in the plant growth enhancement or impairment observed in our study have not been specifically investigated. We believe that maintaining plant nutrients, and especially micronutrients in soluble forms of humic complexes, thereby making them available to the plants (as firstly proposed by Chen and Aviad, 1990), is the major mechanism involved with plant growth enhancement. Hence, we chose to test
HS application in suboptimal nutrient solutions. Our results obtained in the microplate and pouch systems clearly demonstrate that these methods may serve as screening methods for larger tests. Obviously, greenhouse and field experiments are needed for further confirmation of the observed positive effects.

REFERENCES


