Effect of Biochar Amendments on Mycorrhizal Associations and Fusarium Crown and Root Rot of Asparagus in Replant Soils

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Abstract

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Pyrolyzed biomass waste, commonly called biochar, has attracted interest as a soil amendment. A commercial prototype biochar produced by fast pyrolysis of hardwood dust was examined in soils to determine if it could reduce the damaging effect of allelopathy on arbuscular mycorrhizal (AM) root colonization and on Fusarium crown and root rot of asparagus. In greenhouse studies, biochar added at 1.5 and 3.0% (wt/wt) to asparagus field soil caused proportional increases in root weights and linear reductions in the percentage of root lesions caused by *Fusarium oxysporum* f. sp. *asparagi* and *F. proliferatum* compared with a control. Concomitant with these effects was a 100% increase in root colonization by AM fungi at the 3.0% rate. Addition of aromatic acids (cinnamic, coumaric, and ferulic) that are known allelopathic agents affecting asparagus reduced AM colonization but the deleterious effects were not observed following the application of biochar at the higher rate. When dried, ground, asparagus root and crown tissues infested with *Fusarium* spp. were added to soilless potting mix at 0, 1, or 5 g/liter of potting mix and then planted with asparagus, there was a decrease in asparagus root weight and increase in disease at 1 g/liter of potting mix but results were inconsistent at the higher residue rate. However, when biochar was added at 35 g/liter of potting mix (roughly 10%, vol/vol), these adverse effects on root weight and disease were equal to the nontreated controls. A small demonstration was conducted in field microplots. Those plots amended with biochar (3.5% [wt/wt] soil) produced asparagus plants with more AM colonization in the first year of growth but, in the subsequent year, biochar-treated plants were reduced in size, possibly due to greater than average precipitation and the ability of biochar to retain moisture that, in turn, may have created conditions conducive to root rot. These studies provide evidence that biochar may be useful in overcoming the deleterious effects of allelopathic residues in replant soils on asparagus.

Biochar is a charcoal-like carbonaceous byproduct of biomass pyrolysis (25,27). It is produced from crop residue, wood chips, manure, or other wastes at temperatures between 400 and 700°C in the near absence of oxygen. Although the initial interest in biomass pyrolysis was the value of distilled gases and fuels that could be collected, the biochar byproduct itself has gained much more attention as a potentially beneficial soil amendment and as a means of sequestering carbon in a form that can withstand decomposition to CO_2 in soil over centuries. Advocates have argued that there is potential for the annual sequestration of atmospheric CO_2 at the billion-ton scale within 30 years (38). If massive-scale deployment does occur over the next few decades, biochar may be available for widespread use as a soil amendment at levels ranging from a few tenths to several percent of soil by weight in the rhizosphere. However, many claims regarding the usefulness of biochar have not been experimentally validated.

Addition of biochar to soil results in increased nutrient retention and water-holding capacity in soil (5,26). Although some studies have shown positive effects of biochar on yields of maize, several legumes, and several species of trees (28,38), in other studies, biochar either had no value or was harmful toward plants (14). Generalizations about the utility of biochar are confounded by the wide variability in composition, texture, and adsorptive properties of biochar depending on the feedstock, temperature, and other conditions used in its preparation.

Depending on source stock and formation conditions, charcoals can be strongly adsorptive toward organic compounds (4,42). Rotting asparagus crowns release allelopathic toxins, notably aromatic acids such as coumaric, caffeic, and ferulic acids (19,21). Asparagus is also susceptible to Fusarium crown and root rot caused by *Fusarium oxysporum* Schltdl. and *F. proliferatum* (Matsush.) Nirenberg (synanamorph = *F. moniliforme* J. Sheld.). These two factors contribute to the asparagus decline problem (2,11) and to the subsequent replant problem that occurs when old fields are replanted with asparagus (15). Thus, the potential of biochar to adsorb allelochemicals makes it attractive for mitigating the replant problem in asparagus. There have been varying opinions as to whether these allelochemicals actually affect disease severity (1,20,34), It is documented that *Fusarium* spp. are not affected by the allelochemicals, which may allow them to proliferate in the absence of competitors (3). These toxins have been shown to inhibit beneficial microorganisms such as vesicular arbuscular mycorrhizae (AM) (29,33), *Trichoderma* spp., and *Gliocladium* spp. (3), causing reduced plant vigor and increased susceptibility to Fusarium crown and root rot (9). The addition of activated carbon to asparagus soils improved growth and increased AM colonization in greenhouse studies (30). Injecting flowable activated charcoal into the root zones of established plants increased growth and performance in young fields but was less effective in older fields (32). However, in studies on other plants, activated carbon improved plant growth while reducing AM colonization, possibly by improving the availability of phosphorus which, in turn, inhibited AM colonization (41). Increased AM colonization has also been closely associated with the suppression of Fusarium crown and root rot (40) and tolerance to allelopathy (31). Other beneficial microbes such as fluorescent pseudomonads are implicated in the suppression of Fusarium crown and root rot of asparagus and may be a useful indicator of soil and root health (8,10).

The structure and properties of biochar are similar to those of activated carbon in some respects; therefore, it is reasonable to expect that biochar will influence soil biology and affect crop health. However, the mechanisms by which biochar may affect crop health are still obscure. Elad et al. (6) reported that biochar can elicit the systemic acquired resistance pathway in plants and

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provide disease protection against *Botrytis cinerea* (gray mold) and *Leveillula taurica* (powdery mildew) on pepper and tomato.

Biochar amendments may reduce damage from Fusarium crown and root rot of asparagus in replanted asparagus soils. The disease-suppressing mechanism may be associated with increasing AM colonization and mineral nutrition of the plant. Therefore, the objectives of this study were to determine the effect of biochar on asparagus growth, disease, AM colonization, and elemental composition of asparagus in soils where allelochemicals were artificially added.

Materials and Methods

Greenhouse study I. The first greenhouse study examined the effect of biochar on asparagus in soil naturally infested with *Fusarium* spp. and artificially supplemented with allelochemicals. Seed of susceptible 'Mary Washington' were agitated for 1 h in 20% household bleach (0.105% $NaHClO_2$) and rinsed three times with distilled H_2O to eliminate seedborne pathogens. Seed were soaked in distilled water at 30°C for 72 h, germinated in trays with 36 cells filled with potting mix (ProMix BX; Premier Brand Inc., New Rochelle, NY), and held for 10 to 12 weeks in the greenhouse. Seedlings received Hoagland's solution at 50 ml/cell (22) after 4 weeks and were irrigated as needed. Seedlings were removed and the soil was washed from the roots. Seedlings with no root lesions that weighed between 5 and 7 g were selected for the experiments. Soil (pH 6.4) for the experiment was removed from a field where asparagus had been grown for many years. Soil was shaken loose from exhumed asparagus root systems, air dried, and passed through a 0.5-cm sieve. The soil contained small pieces of asparagus roots. Serial dilution onto selective agar revealed *Fusarium* spp. at approximately 5×10^3 CFU/g of soil but the actual fraction capable of causing disease was not determined. Half of the soil was autoclaved at 121°C for 1 h. The autoclaved or nonautoclaved batches were mixed 1:1 with autoclaved sand (bulk density = 1.25), amended with biochar (CQuest Biochar; Dynamotive Energy Systems Corp., McLean, VA) at the rate of 1.5 and 3.0% (wt/wt), and then placed into 10-cm plastic pots (350 cm^3). Each pot contained 440 g of soil mix. The manufacturer's analysis of CQuest Biochar revealed that it was composed of 74% C, 3.2% H, 11.2% O, and 11% ash that may have provided small amounts of essential nutrients. Soil not treated with biochar served as a control. To ensure that AM fungi were present in sufficient densities, endomycorrhizal inoculant (BEI Bio/Organics, La Pine, OR), which contained spores of *Glomus brasilianum* Spain & J. Miranda, *G. clarum* T.H. Nicolson & N.C. Schenck, *G. deserticola* Trappe, Bloss & J.A. Menge, *G. intraradices* N.C. Schenck & G.S. Sm., *G. monosporum* Gerd. & Trappe, *G. mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe, and *Gigaspora margarita* W.N. Becker & I.R. Hall, was mixed into the soil at 1 g/liter of soil mix. One 12-week-old asparagus transplant was planted into each pot. The next day, each pot was supplemented with 50 ml of distilled water containing 0, 5.0, or 50.0 µg of caffeic, coumaric, and ferulic acids per milliliter (equivalent to 0, 0.57, or 5.7 mg of each acid per gram of soil). Acids were initially dissolved by heating at 60°C in a small amount of ethanol and brought to volume with deionized water (final liquid composition, 2.0% ethanol by volume). Plants were grown at 15 to 20°C (night) and 20 to 25°C (day) under sodium vapor lamps set for 12-h-day, 12-h-night photoperiods. Plants were irrigated as needed and fertilized twice a month with 50 ml of Hoagland's solution per pot. This experiment consisted of 18 treatments (three [biochar rates] × two [infested or autoclaved] × three [allelochemical concentrations]) with 12 replicates per treatment. Pots were set on greenhouse benches in a randomized blocked design (three blocks, four replicates/block). The experiment was repeated the following year.

After 12 weeks, plants were removed from the pots and the soil was shaken off. Rhizosphere soil was sampled by shaking each root system into a plastic bag and refrigerating it at 4°C. Roots were then washed with tap water to remove soil, and the fresh weights of the ferns and roots plus crown were recorded. Fern tissue was dried and reweighed. Tissue from plants grown in autoclaved soil was used in tissue analyses described below. Root systems were divided in half. One half was assayed for disease as described below, while the other half was placed in formalin-acetic acid-alcohol (FAA) (35) until the root systems could be assayed for AM colonization. Feeder roots (1 to 2 cm long) were assayed using modifications of Phillips and Haymans (35) as described by Elmer (9). Between 150 and 200 intersects were counted from each root system, scored as colonized or not, and expressed as a percentage of the total intersects colonized by AM. Disease severity (percentage of roots with lesions) and colonization by *Fusarium* spp. (CFU per centimeter of root) were determined as described previously (7). Rhizosphere soils were assayed for total bacteria, fluorescent pseudomonads, and total *Fusarium* populations as described previously (10) and expressed as log CFU per gram of soil (over dry weight equivalent).

Dried fern samples from each block were bulked and treated as replicates. Samples were ground in a Wiley mill and passed through a 20-mesh sieve. The dried plant tissue was analyzed for total nitrogen by combustion using a LECO FP-528 nitrogen Analyzer (FP-528; Leco Corp., St. Joseph, MI). For analysis of elements K, P, Ca, Mg, S, Fe, Mn, Zn, Cu, and B, dried plant tissue (0.5 g) was digested in 50-ml polypropylene digestion tubes with 5 ml of concentrated nitric acid at 115°C for 45 min using a hot block (DigiPREP System; SCP Science, Champlain, NY). The digested samples were diluted to a 50-ml volume with distilled deionized water. Digested plant tissues were analyzed for the nutrient elements by inductively coupled plasma atomic emission spectroscopy (iCAP 6500; Thermo Fisher Scientific, Waltham, MA).

Greenhouse study II. A second study examined the effect of biochar on Fusarium crown and root rot in soilless potting mix amended with increasing amounts of dried *Fusarium* spp.-infested asparagus residues. Asparagus roots and crowns collected above were air dried, blended in a Waring blender for 30 s, and passed through a 0.5-cm sieve. Dried ground asparagus crowns and roots were also incorporated into biochar-amended potting mix and the nonamended mix at the rate of 0, 1, and 5 g of residues per pot. Asparagus plants were transplanted into potting mix (ProMix BX; Premier Brand, New Rochelle, NY) supplemented with or without CQuest Biochar at 3.5 g/liter of potting mix. After 12 weeks, plants were removed, washed, and weighed, and the roots were assayed for disease severity as described above. There were 12 replicate plants per treatment combination, and the experiment was repeated the following year.

Field demonstration. A field demonstration was established in Hamden, CT (sandy loam soil, 1% organic matter) in 2008 to test the practical application of biochar on asparagus growth. Eighteen black plastic pots with five drainage holes (0.45 m in diameter by 0.35 cm deep) each were set into soil 1 m apart in a row. The experiment consisted of three treatments: non-asparagus soil (healthy control), asparagus soil containing 5% asparagus residues, and asparagus soil containing 5% asparagus residues with biochar (3.5% [wt/wt], 10% [vol/vol], approximately 150 metric tons/ha mixed 35 cm deep). There were six replicates per treatment. Asparagus residues were obtained from recently dug crowns that had been chopped into 5- to 10-cm pieces. Microplots were planted with 1-year-old crowns ('Mary Washington') and fertilized with N-P-K fertilizer at 50 kg/ha.

In August 2008 and 2009, asparagus stems were rated by size: small (0.1 to 0.5 cm in diameter), medium (0.6 to 1.0 cm in diameter), and large (>1.0 cm in diameter), and counted. In August 2009, roots were sampled from each microplot by removing soil cores (22.5 by 3 m in diameter) with a soil auger. Five soil cores per microplot were removed approximately 12 to 15 cm from the crown and bulked. Roots were extracted from the soil cores by passing the soil through a 2.8-mm sieve with a slow stream of water and collecting the roots with forceps. Roots were washed in tap water, fixed in FAA, and assayed for mycorrhizae as described above. Marketable yield (22 cm) was harvested three times weekly for 3 weeks in spring 2010.

Statistical methods. Data were subjected to analysis of variance and means were separated using Tukey's test at $P = 0.05$. Percent data were transformed to arcsine of the square root before analysis to achieve homogeneity of variance. Regression analyses were done where appropriate.

Results

Greenhouse study I. The effects of biochar on asparagus root weight and root lesions in *Fusarium* spp.-infested asparagus soil are shown in Figure 1. No significant effects of the allelochemicals were found. The results in Figure 1, therefore, represent combined data from both trials, given that no interactions were noted between the treatment and the two trials. The addition of biochar up to 3% (wt/wt) increased asparagus root weight and suppressed disease in the infested soil, both approximately linearly with biochar rate. Biochar also improved root weight in the autoclaved soil when added up to 1.5% (wt/wt) but further changes at 3% biochar rate were not significantly different (Fig. 1).

Biochar had a positive, linear effect on percent root colonization by AM, independent of whether allelochemicals were added at 5.7 µg/g of soil (Fig. 2). Allelochemicals applied at 5.7 µg/g of soil significantly suppressed root colonization by AM compared with the soil without biochar ($P = 0.03$). Allelochemicals applied at a lower rate (0.57 µg/g of soil) also reduced AM colonization compared with the soil without biochar but the reduction was not significant (*data not shown*). When biochar was added at 1.5 or 3.0%, the allelochemicals had no statistically significant impact on AM colonization.

The rhizosphere pH increased slightly with biochar rate but, because replicates were bulked, it was not possible to determine whether or not trends were statistically significant (Table 1). When compared with the control, rhizosphere densities of fluorescent pseudomonads were significantly increased at the higher biochar rate but not at the lower rate. The density of *Fusarium* spp. in the rhizosphere samples (Table 1) and the bulked soil samples (*data not shown*) was unaffected by biochar rate or allelochemicals.

Biochar amendment was associated with increases in K, S, Mn, and B and with reductions in N, Mg, and Fe (Table 2). The allelochemicals decreased N and P and increased Ca, S, and B. Significant interactions between biochar and the allelochemicals were occasionally observed and were the result of unexpected peaks of S, Mn, Zn, and Cu in the tissue of plants treated with the lower rate (0.57 µg/g of soil) of the allelochemicals that was not observed at higher rate of biochar.

Greenhouse study II. In the second set of experiments, the effect of biochar added at constant rate (0 or 3.5 g/liter) was examined for impact on disease and growth of asparagus grown in soilless potting mix supplemented with variable amounts of dried and ground asparagus residues. This experiment was repeated and significant interactions between treatments and experimental repetition prevented the two data sets from being combined. In the first trial, root weights declined with increasing asparagus residues but these deleterious effects were not observed when biochar was added (Fig. 3A). The lowest residue rate (1 g/liter of potting mix) was associated with the highest amount of root lesions, and biochar significantly reduced the disease at this rate. The repetition of this experiment produced plants that were much smaller in size than in the first study (Fig. 3B). The lowest residue rate (1 g/liter of potting mix) produced the smallest plants but the effect was not observed when residues were added at the higher rate (5 g/liter of potting mix), where an unexpected increase in root weights was observed. The inclusion of biochar produced plants that did not differ from nontreated control plants. As before, the percentage of roots with lesions was highest at the lowest residue rate (43%; 1 g/liter of potting mix), and plants grown with biochar at this same rate did not differ from the control (12%; Fig. 3B).

Microplot demonstration. There was no AM colonization in the sampled roots from non-biochar-treated asparagus soils; however, a low amount (4.4%) was detected in the biochar-treated soils in 2009. In the healthy non-asparagus soil, AM colonization had risen to 14%. Stand counts during summer 2009 revealed no differences in vigor between the treatments but there was a downward trend for the microplots treated with biochar (Table 3). Spear yield recorded in 2010 was similar to stand count data for the three treatments but there were no significant differences for any of the yield components. However, it was noted in July 2010 that three of six replicate microplots treated with biochar had died as opposed to only one microplot of the infested control. The average rainfall

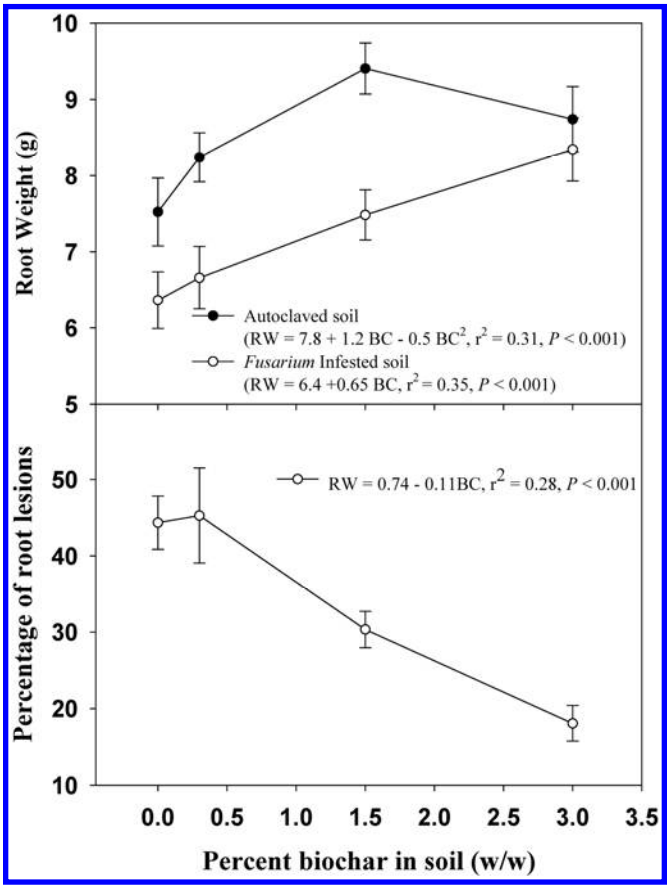

Fig. 1. Effect of biochar rate on root weights or roots with lesions of asparagus grown in autoclaved asparagus soil or asparagus soil naturally infested with *Fusarium oxysporum* f. sp. *asparagi* and *F. proliferatum.* Error bars represent the standard error of the means (n = 20) from two combined trials.

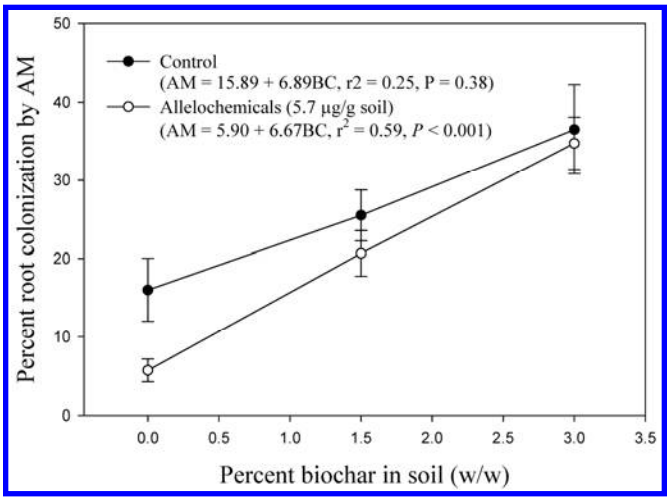

Fig. 2. Effect of biochar rate on colonization of asparagus roots by arbuscular mycorrhizae (AM) grown in soil that was spiked or not with an allelochemicals mixture (caffeic, coumaric, and ferulic acids, each acid at 5.7 µg/g of soil). Error bars represent the standard error of the means from two combined experiments.

during the period of fern production (June and July) in 2009 was excessive (36.2 cm) compared with the past 10 years, which averaged 21.0 cm for the same time period. Biochar-treated microplots were noted to hold water longer and dried down more slowly than untreated microplots.

Discussion

Failure of asparagus to establish in abandoned asparagus fields is attributed to several factors, one of which is the release of aromatic acids and other allelochemicals from plant residues. The main toxins are the aromatic acids: coumaric, caffeic, and ferulic acids (19). These toxins make plants more susceptible to Fusarium crown and root rot disease (34). Given that the removal of these residues remains impractical, our objectives were to determine whether biochar could be useful to overcome the replant problem. Addition of the above aromatic acids to the soil in the current study was an attempt to reproduce the conditions found in a replant situation. The aromatic acids had no effect on the percentage of root lesions or on root weights but they did reduce root colonization by AM. In previous studies, filtrates of asparagus residues containing these compounds inhibited growth of asparagus (1,21) but it is likely that the filtrates contained other toxins. In fact, in greenhouse study II, root weight was negatively affected by ground dried asparagus residues added to soilless potting mix. In both repetitions of that study, disease was unexpectedly greatest at the lowest residue rate.

In the first set of greenhouse experiments, most growth and disease responses were proportional to the biochar rate but thresholds were occasionally observed at the lower rate, suggesting that biochar interactions with soil properties may exist. A clear reduction in the percentage of root lesions caused by *Fusarium* spp. was observed following the addition of biochar to soil along with an increase in AM colonization. Both Wacker et al. (40) and Matsurba et al. (31) observed that disease suppression was closely associated with an increase in AM colonization. The current study supports that observation and may suggest that increased AM colonization may suppress infection and disease development in asparagus (9).

The present study is in qualitative agreement with Matsubara et al. (30), who found coconut charcoal amendments suppressed Fusarium crown and root rot and increased AM colonization of asparagus seedlings (from 32 to 55%). However, the conditions of that study and the current one differ enough that different mecha-

Table 1. Soil pH and densities of *Fusarium* spp. and fluorescent pseudomonads associated with rhizosphere asparagus roots treated with allelochemicals and grown in soil amended with different rates of biochar[y]

Treatment	Rhizosphere pH	*Fusarium* counts (log CFU/g of soil)	Fluorescent pseudomonads (log CFU/g of soil)
No biochar			
No allelochemicals	6.87	3.72	5.60
Allelochemicals (5.7 µg/g)	7.11	3.86	5.74
Mean	6.99	3.80	5.67
Biochar 1.5% (wt/wt)			
No allelochemicals	7.24	4.29	5.44
Allelochemicals (5.7 µg/g)	7.19	3.88	5.45
Mean	7.22	4.13	5.45
Biochar 3.0 (wt/wt)			
No allelochemicals	7.26	3.80	5.97
Allelochemicals (5.7 µg/g)	7.27	3.56	5.95
Mean	7.27	3.70	5.96
ANOVA for tissue, source *P*			
Biochar	ns[z]	ns	0.008
Allelochemicals	ns	ns	ns
Biochar × allelochemicals	ns	ns	ns

[y] Abbreviations: ANOVA = analysis of variance and ns = not significant.
[z] Significant at $P = 0.078$.

Table 2. Mineral composition of asparagus ferns treated with allelochemicals and grown in soil amended with biochar[w]

	Minerals (µmol/g of tissue)										
Treatment[x]	N[y]	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
No biochar											
No alleo.	1.37	65	816	114	68	69	1.76	0.32	0.26	0.08	3.62
Allelo. 0.57 µg/g	1.31	60	860	127	68	73	1.35	0.35	0.22	0.08	4.32
Allelo. 5.7 µg/g	1.32	63	865	112	62	67	1.43	0.35	0.28	0.08	3.87
Mean	1.33	63	847	118	66	70	1.51	0.34	0.25	0.08	3.94
Biochar 1.5% (wt/wt)											
No alleo.	1.31	65	879	107	63	70	1.48	0.37	0.23	0.07	4.14
Allelo. 0.57 µg/g	1.19	58	926	118	63	71	1.26	0.36	0.27	0.08	4.33
Allelo. 5.7 µg/g	1.06	59	853	121	65	69	1.32	0.39	0.24	0.09	4.67
Mean	1.19	61	886	115	63	70	1.35	0.38	0.25	0.08	4.38
Biochar 3.0 (wt/wt)											
No alleo.	1.27	65	905	111	58	72	1.16	0.37	0.24	0.09	4.24
Allelo. 0.57 µg/g	1.19	62	925	119	59	74	1.24	0.45	0.26	0.09	5.03
Allelo. 5.7 µg/g	1.07	56	891	101	57	66	1.09	0.37	0.24	0.06	3.94
Mean	1.18	61	907	110	58	70	1.16	0.39	0.25	0.08	4.40
ANOVA, source *P*											
Biochar	*[z]	ns	0.001	ns	0.001	0.023	0.0	0.001	ns	ns	0.046
Allelo.	0.001	0.023	ns	0.024	ns	0.01	0.03	ns	ns	ns	0.003
Biochar × allelo.	ns	ns	ns	ns	ns	0.001	ns	0.049	0.03	0.02	ns

[w] Values represent means of six replicates; three bulked samples from each experimental repetition; Alleo. = allelochemicals, ANOVA = analysis of variance for tissue, and ns = not significant.
[x] A 50-ml mixture of allelochemicals containing caffeic, coumaric, and ferulic acids at 0, 5.0, or 50.0 µg/ml was applied to 440 g of soil.
[y] Nitrogen concentrations are expressed a mmol/g tissue.
[z] Significant at $P = 0.062$, Kruskal Wallace test at $P = 0.025$.

nisms may be operative. No allelopathy was imposed in the Matsubara et al. (30) study; therefore, the increase in AM colonization and disease suppression observed may have been due to other effects, such as changes in soil structure that favor survival of the AM fungi. Moreover, soil pH in their study was acidic (pH 5.4) and rose to pH 6.3 following the addition of coconut charcoal. Although pH was not considered to be important in affecting germination of the AM fungi, the alkalization effect of their soils may have suppressed Fusarium disease severity and promoted root health and beneficial microbes, which, in turn, increased AM colonization.

Soil nutrient levels were not measured in the present study but it has been documented that biochar enhances nutrient retention and water-holding capacity of soil (5,26) along with supplying a small amount of nutrients. This may explain the growth-promoting effects observed in autoclaved soil where disease was minimized. Another possible mechanism for growth promotion is the possible production of ethylene, a plant hormone, from biochar amendment (39). Ethylene at concentrations less than 2 ppm can increase AM germination and hyphal growth (23). The evolution of ethylene from biochar and its role on root growth remains an interesting mechanism to be investigated.

Warnock et al. (41) discussed several ways that biochar could affect AM colonization in plants: (i) alteration of nutrient availability or soil properties, (ii) stimulation of soil microbial populations that favor AM colonization, (iii) disruption of chemical signaling or detoxification of allelochemicals that inhibit AM colonization, and (iv) creation of a physical refuge from AM predators. Although the present study was not designed to elucidate the relative importance of the aforementioned mechanisms, there is evidence that biochar may function through at least two of them: alteration of nutrient availability and stimulation of soil microbes that favor AM colonization.

Increasing biochar rate increased K, S, Mn, and B uptake whereas N, Fe, and Mg uptake decreased. Analysis of the CQuest biochar revealed that all of these elements except B were present in the biochar; however, because the choice of procedures to estimate elemental composition of biochar in soil can drastically affect the results (J. Lehman, Cornell University, *personal communication*), the actual amount available to plants is difficult to predict. The

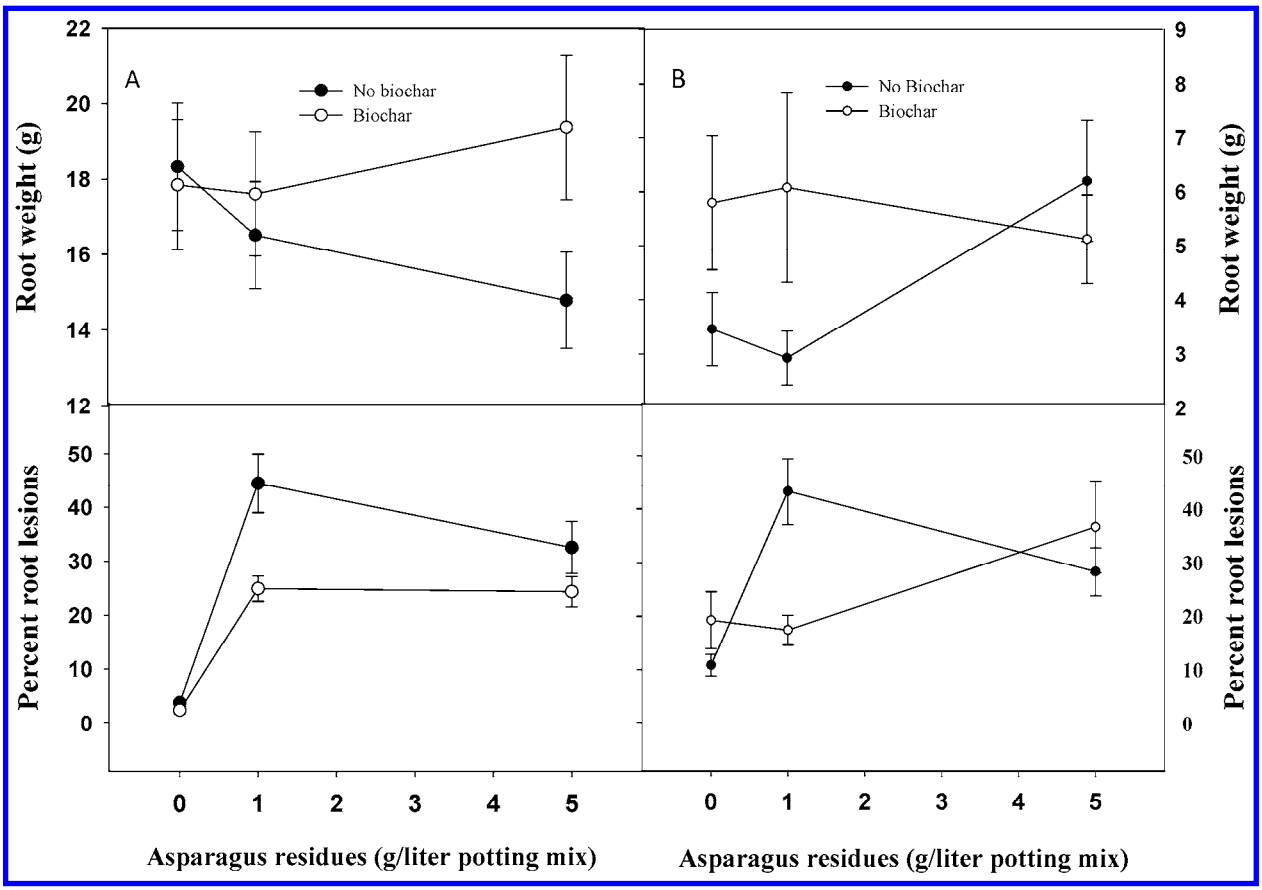

Fig. 3. Effect of increasing rates of dried asparagus residues in soilless potting mix amended with and without biochar (3.5 g/litter of potting mix) on root weight and the percentage of roots with lesions caused by *Fusarium oxysporum* f. sp. *asparagi* and *F. proliferatum.* **A,** Experiment 1 and **B,** experiment 2. Error bars represent the standard error of the means (n = 20).

Table 3. Stand counts, marketable yield, and arbuscular mycorrhizal (AM) colonization of asparagus roots from microplots treated with biochar[x]

	Marketable yield				
Treatments[y]	**Number of spears**	**Spear weight (g)**	**Stand counts**	**Disease rating (1–5)[z]**	**AM colonization (%)**
Control (CK)	2.1 a	19.4 a	5.7 a	3.0 a	0.0
Biochar	1.3 a	17.1 a	5.0 a	2.5 ab	4.4
Healthy CK	3.3 a	45.0 b	5.7 a	1.9 b	14.1

[x] Values represent the means of six replicates; values followed by differing letter are significant different by Tukey's test at P = 0.05; AM colonization data represent one bulked sample from six replicate plots.
[y] Control = untreated asparagus soil, Biochar = asparagus soil treated with biochar at 10% (vol/vol) (3.5% wt/wt), untreated non-asparagus soil.
[z] Disease rating based on the scale 1 = green and robust ferns, 2 = slightly less vigorous ferns, 3 = yellowing in the fern tissue, 4 = yellow and wilts, or 5 = dead or near dead.

reduction in N uptake was unexpected because the root systems were larger in the biochar-amended pots. Potassium and S are active in host defense metabolism by influencing osmotic relations and the production of defense products (18,36), and the increased uptake of these elements may have contributed to disease suppression. The increase in Mn levels is interesting, given past research associating disease suppression with an increase in the availability of Mn in asparagus following NaCl application to soils (8,10) and in surveys associating low Mn in field soils where Fusarium crown and root rot was severe (17). Past studies on asparagus (7) found disease suppression to be associated with a reduction in Mg in the fern tissue, also observed in the current study. In addition, the slight increase in soil pH may have contributed to reduced disease by decreasing the availability of Fe to the pathogen (24). The linear reduction in Fe concentration in the asparagus tissue with biochar concentration in the soil (Table 2) lends support to this mechanism. These nutrient alterations may have promoted a more disease-resistant plant. In other studies, biochar applied at 20 t/ha (which approximates 1% [wt/wt] in the top 15 cm of soil) increased the nutrient-holding capacity of the soil, boosted the yield of maize, and increased tissue levels of Ca and Mg (28). Lehmann et al. (26) reported that biochar amendment to tropical soils initially increased K, P, and Zn availability and, to a lesser extent, Ca and Cu. The differences in soil types and the different types of biochar used in these studies make comparisons difficult.

Biochar also increased the density of beneficial fluorescent pseudomonads. Esfehani et al. (13) found that fluorescent pseudomonads alone could enhance mycorrhizal colonization in wheat. In addition, competition, antibiosis, and induced resistance are well-documented methods by which fluorescent pseudomonads can suppress disease and increase root health (16). Elad et al. (6) suggested that biochar induced resistance to *Botrytis* spp. and powdery mildew on pepper and tomato.

The effect of biochar on adsorption or detoxification of toxins was not examined in the current study but data are forthcoming. Similarly, the fourth mechanism proposed by Warnock suggests that biochar can provide refuge of AM propagules from predation. Matsubara et al. (29) and Saito (37) both suggested that carbonized material could enhance the growth of AM fungi in soil by providing optimum air and water permeability while excluding antagonists. This hypothesis also needs validation.

The water-holding capacity of biochar-treated soils may offer potential benefits in nutrient-poor sandy soils that are prone to moisture deficits. Asparagus is relatively drought tolerant but moisture deficits can promote disease (12). In heavier clay soils, it is conceivable that biochar may be a detriment to root health by promoting root rot. This may explain the poor asparagus growth we observed in microplots on loam soil. Studies are underway to examine the role of biochar on asparagus grown in sandy soils.

Based on these studies, there appears to be a potential role for biochar in the asparagus replant problem. Its applicability to other replant problems on apple and nut trees needs to be determined. However, until more is known about how biochar rates and different biochar types interact in different soils and on different crops, caution should be exercised in making any specific endorsements of the use of biochar for disease suppression.

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Literature Cited

1. Blok, W. J., and Bollen, G. J. 1993.The role of autotoxins from root residues from the previous crop in the replant disease of asparagus. Neth. J. Plant Pathol. 99 (Suppl. 3):29-40.
2. Blok, W. J., and Bollen, G. J. 1996. Etiology of asparagus replant bound early decline. Eur. J. Plant Pathol. 102:87-98.
3. Blok, W. J., and Bollen, G. J. 1996. Interactions of asparagus root tissue with soil microorganisms as a factor in early decline of asparagus. Plant Pathol. 45:809-822.4
4. Braida, W., Pignatello, J. J., Lu, Y., Ravikovitch, P. I., Neimark, A. V., and Xing, B. 2003. Sorption hysteresis of benzene in charcoal particles. Environ. Sci. Technol.37:409-417.
5. Chan, K. Y., Van Zwieten, L., Meszaros, I., Downie, A., and Joseph, S. 2007.Agronomic values of greenwaste biochar as a soil amendment. Aust. J. Soil Res.45:629-634.
6. Elad, Y., Rav David, D., MellerHarel, Y., Borenshtein, M., Ben Kalifa, H, Silber, A., and Graber, E. R. 2010. Induction of systemic resistance in plants by biochar, a soil-applied carbon sequestering agent. Phytopathology100:913-921.
7. Elmer, W. H. 1992. Suppression of Fusarium crown and root rot of asparagus with sodium chloride. Phytopathology 82:97-104.
8. Elmer, W. H. 1995. The association among Mn-reducing bacteria and sodium chloride applications in the suppression of Fusarium crown and root rot of asparagus. Phytopathology 85:1461-1467.
9. Elmer, W. H. 2002. Influence of formononetin and NaCl on mycorrhizal colonization and Fusarium crown and root rot of asparagus. Plant Dis. 86:1318-1324.
10. Elmer, W. H. 2003. Local and systemic effects of NaCl on root composition, rhizobacteria, and Fusarium crown and root rot of asparagus. Phytopathology 93:186-195.
11. Elmer, W. H., Johnson, D. A., and Mink, G. I. 1996. Epidemiology and management of the diseases causal to asparagus decline. Plant Dis. 80:117-125.
12. Elmer, W. H., and LaMondia, J. A. 1999. Studies on the suppression of Fusarium crown and root rot with NaCl. Acta Hortic. 479:211-218.
13. Esfehani, Y. J., Khavazi, K., and Ghorbani, S. 2009. Cross interaction of *Pseudomonas putida* and *Glomus intraradices* and its effect on wheat root colonization. Pak. J. Biol. Sci. 12:1365-1370.
14. Glaser, B., Lehmann, J., and Zech, W. 2002.Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—a review. Biol. Fertil. Soils 35:219-230.
15. Grogan, R. G., and Kimble, K. A. 1959. The association of Fusarium wilt and root rot with the asparagus decline and replant problem in California. Phytopathology 49:122-125.
16. Haas, D., and Défago, G. 2005. Biological control of soilborne pathogens by fluorescent pseudomonads. Nat. Rev. Microbiol. Online. doi: 10.1038/nrmicro1129.
17. Hamel, C., Vujanovic, V., Nakano-Hylander, A., Jeannotte, R., and St.-Arnaud, M. 2005. Factors associated with Fusarium crown and root rot of asparagus outbreaks in Quebec. Phytopathology 95:867-873.
18. Haneklaus, S., Bloem, E., and Schnug, E. 2007, Sulfur and plant disease. Pages 101-118 in: Mineral Nutrition and Plant Disease. L. E. Datnoff, W. H. Elmer, and D. M. Huber, eds. American Phytopathological Society, St. Paul, MN.
19. Hartung, A., Nair, M., and Putnam, A. 1990. Isolation and characterization of phytotoxic compounds from *Asparagus officinalis*. J. Chem. Ecol. 16:1707-1733.
20. Hartung, A. C., and Stephens, C. T. 1983.Effects of allelopathic substances produced by asparagus on the incidence and severity of asparagus decline due to Fusarium crown rot. J. Chem. Ecol. 9:1163-1174.
21. Hazelbrook, J. P., Garrison, S. A., and Gianfagna, T. 1989. Allelopathic substances in asparagus roots: extraction, characterization, and biological activity. Am. Soc. Hortic. Sci. 114:152-158.
22. Hoagland, D. R., and Arnon, D. I. 1938.The water culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347.
23. Ishii, T., Shrestha, Y. H., Matsumoto, I., and Kadoya, K. 1996. Effect of ethylene on the growth of vesicular arbuscular mycorrhizal fungi and on the mycorrhizal formation of trifoliate orange roots. J. Jpn. Soc. Hortic. Soc 65:525-529.
24. Jones, J. P., Engelhard, A. W., and Woltz, S. S. 1989. Management of Fusarium wilt of vegetables and ornamentals by macro- and microelement nutrition. Pages 18-32 in: Soilborne Plant Pathogens: Management of Diseases with Macro- and Microelements. A. W. Engelhard. ed. American Phytopathological Society, St. Paul, MN.
25. Lehmann, J., da Silva, J. P., Jr., Steiner, C., Nehls, T., Zech, W., and Glaser, B. 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant Soil 249:343-357.
26. Lehman, J. 2007.A handful of carbon. Nature 447:143-144.
27. Lehmann, J., and Joseph, S., eds. 2009. Biochar for Environmental Management: Science and Technology. Earthscan Publications, Ltd., London.
28. Major, J., Rondon, M., Molina, D., Riha, S. J., and Lehmann, J. 2010. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. Plant Soil. Online. doi: 10.1007/s11104-010-0327-0
29. Matsubara, Y., Harada, T., and Yakuwa, T. 1995.Effect of inoculation density on vesicular arbuscular mycorrhizae fungal spores and addition of carbonized materials to bed soil on growth of Welsh onion seedlings. J. Jpn. Soc. Hortic. Sci. 64:549. (In Japanese with English summary)
30. Matsubara, Y., Hasegawa, N., and Fukui, H. 2002. Incidence of Fusarium root rot in asparagus seedlings infected with arbuscular mycorrhizal fungus as affected by several soil amendments. J. Jpn. Soc. Hortic. Sci. 71:370-374.
31. Matsubara, Y., Okada, T., and Nahiyan, A. S. M. 2010. Tolerance to allelo-

pathy and Fusarium disease, changes in antioxidant substance in mycorrhizal asparagus plants raised in decline soil. Acta Hortic. 883:417-423.
32. Motoki, S., Hattori, T., and Oka, J. 2008. Allelopathy in asparagus 2: effect of injection period and concentration on deep placement method of activated charcoal flowable in growing period of asparagus. Acta Hortic. 776:91-104.
33. Pedersen, C. T., Safir, G. R., Siqueira, J. O., and Parent, S. 1991. Effect of phenolic compounds on asparagus mycorrhiza. Soil Biol. Biochem. 3:491-494.
34. Peirce, L. C., and Colby, L. W. 1987. Interaction of asparagus root filtrates with *Fusarium oxysporum* f. sp. *asparagi*. Am. Soc. Hortic. Sci. 112:35-40.
35. Phillips, J. M., and Haymans, D. S. 1970. Improved procedure for clearing root and counting parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158-163.
36. Prabhu, A., Fageria, N., Huber, D. M., and Rodrigues, F. 2007. Potassium and plant disease. Pages 57-78 in: Mineral Nutrition and Plant Disease. L. E. Datnoff, W. H. Elmer, and D. M. Huber, eds. American Phytopathological Society, St. Paul, MN.
37. Saito, M. 1990. Charcoal as a micro habitat for VA mycorrhizal fungi, and its practical application. Agric. Ecosyst. Environ. 29:341-344.
38. Sohi, S., Lopez-Capel, E., Krull, E., and Bol, R. 2009. Biochar, climate change and soil: a review to guide future research. CSIROL and Water Science Report Series ISSN: 1834-6618.
39. Spokas, K. A., Baker, J. M., and Reicosky, D. C. 2010. Ethylene: potential key for biochar amendment impacts. Plant Soil. Online. doi: 10.1007/s11104-010-0359-5
40. Wacker, T. L., Safir, G. R., and Stephens, C. T. 1990. Mycorrhizae fungi in relation to asparagus growth and Fusarium wilt. Acta Hortic. 271:417-422.
41. Warnock, D. D., Lehmann, J., Kuyper, T. W., and Rillig, M. C. 2007.Mycorrhizal responses to biochar in soil—concepts and mechanisms. Plant Soil 300:9-20.
42. Zhu, D., and Pignatello, J. J. 2005. Characterization of aromatic compound sorptive interactions with black carbon (charcoal) assisted by graphite as a model. Environ. Sci. Technol.39:2033-2041.