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REVIEW

The Biochar Effect: plant resistance to biotic stresses

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Summary. Biochar (charcoal) is the solid co-product of pyrolysis, the thermal degradation of biomass in the absence of oxygen. Pyrolysis also yields gaseous and liquid biofuel products. There is a growing interest worldwide in the pyrolysis platform, for at least four reasons: (i) pyrolysis can be a source of renewable biofuels; (ii) many biomass waste materials can be treated by pyrolysis and thus converted into a fuel resource; (iii) long-term sequestration of carbon dioxide which originated in the atmosphere may result from adding biochar to soil, and (iv) biochar soil amendment contributes to improved soil fertility and crop productivity. Currently, however, very little biochar is utilized in agriculture, in part because its agronomic value in terms of crop response and soil health benefits have yet to be quantified, and because the mechanisms by which it improves soil fertility are poorly understood. The positive effects of biochar on crop productivity under conditions of extensive agriculture are frequently attributed to direct effects of biochar-supplied nutrients and to several other indirect effects, including increased water and nutrient retention, improvements in soil pH, increased soil cation exchange capacity, effects on P and S transformations and turnover, neutralization of phytotoxic compounds in the soil, improved soil physical properties, promotion of mycorrhizal fungi, and alteration of soil microbial populations and functions. Yet, the biochar effect is also evident under conditions of intensive production where many of these parameters are not limited. Biochar addition to soil alters microbial populations in the rhizosphere, albeit via mechanisms not yet understood, and may cause a shift towards beneficial microorganism populations that promote plant growth and resistance to biotic stresses. In addition to some scant evidence for biochar-induced plant protection against soilborne diseases, the induction of systemic resistance towards several foliar pathogens in three crop systems has been demonstrated. There are indications that biochar induces responses along both systemic acquired resistance (SAR) and induced systemic resistance (ISR) pathways, resulting in a broad spectrum controlling capacity in the canopy. This review examines the effects of biochar soil amendment on the different soil-plant-microbe interactions that may have a role in plant health. Improvement of plant responses to disease can be one of the benefits gained from applying biochar to soil.

Key words: plant pathogen, rhizosphere, microflora, soil-borne disease, PGPR.

What is biochar?

The first evidence of humans' deliberate use of charcoal shows it was utilized as a fuel at least 5500 years ago in Southern Europe and the Middle East. By the commencement of the Bronze Age in Britain around 4000 years ago, the use of charcoal as a metallurgical fuel was commonplace. Yet, fuel was not the only ancient use for charcoal. There is considerable evidence that pre-Columbian natives of the Amazon Basin used charcoal as a soil additive together with manures, bones and pottery shards, turning otherwise unproductive

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soils into rich and fertile ones (Sombroek, 1966). One of the major reasons these "Terra Preta" soils, abandoned between 500 to 2500 years ago, are fertile even today has been attributed to the nutrient-holding capacity of the added charcoal (Smith, 1980). Similar scattered pockets of ancient, fertile, charcoal-containing anthrosols amidst native low fertility soils have been since found in parts of Ecuador, Peru, Western Africa, South Africa, Australia, and Asia. An example of charcoal use in Asian agriculture more than 300 years ago has been preserved in a textbook entitled 'Nogyo Zensho' (Encyclopedia of Agriculture) written by Yasusada Miyzaki in 1697, and translated thusly by Ogawa and Okimori (2010): "After charring all waste, concentrated excretions should be mixed with it and stocked for a while. When you apply this manure to the fields, it is efficient for yielding any crop."

As evidenced in 19th and early 20th century agronomy literature, it appears that charcoal also enjoyed widespread use in North American and European agriculture and horticulture. Some of the uses of charcoal were described in 'A Brief Compend of American Agriculture' by R.L. Allen (1847):

> *Charcoal dust [drilled in with the seed] has been found to increase the early growth from four to ten-fold (p. 150).*
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> *Scattered over the ground … [charcoal] absorbs and condenses the nutritive gases within its pores, to the amount of from 20 to over 80 times its own bulk. ... Charcoal … often checks rust in wheat, and mildew in other crops; and in all cases mitigates their ravages, where it does not wholly prevent them (p. 45).*
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> *A dressing of charcoal has in many instances, been found an adequate preventative [of rust]; and so beneficial has it proved in France, that it has been extensively introduced there for the wheat crop (p. 109).*

The use of charcoal in agriculture waned considerably in the 20th century, presumably due to its increased value as fuel and with the development of modern chemical fertilizers and pest control products. However, since the opening years of the 21st century, there has been a remarkable resurgence of interest worldwide in the agricultural utilization of charcoal for at least four inter-related reasons: (i) Pyrolysis, the means by which charcoal is produced, generates renewable energy products. It is thought that pyrolysis (thermal degradation of organic biomass in the absence of oxygen) may become part of an arsenal of affordable renewable energy technologies aimed at reducing net greenhouse gas emissions from the burning of fossil fuels, and at diversifying energy supplies. (ii) Many organic wastes can be treated and converted into energy via pyrolysis. As a result, pyrolysis is more versatile than technologies that produce biodiesel and ethanol from crops, and does not compete for resources with food production. Many different urban, agricultural and forestry biomass wastes and residues can be treated by pyrolysis. (iii) When used as a soil conditioner together with organic and inorganic fertilizers, charcoal appears to significantly improve soil tilth, productivity, nutrient retention and availability to plants, improved water holding capacity, and soil aggregate stability (Glaser *et al.*, 2002). Because it aids in soil retention of nutrients and agrochemicals for plant and crop utilization (Steiner *et al.*, 2007; Steiner *et al.*, 2008b), charcoal amendment may help fight against soil degradation, and can be a tool in the creation of sustainable food and fuel production in areas with severely depleted soils, scarce organic resources, and inadequate water and chemical fertilizer supplies. (iv) The half-life of biochar in soil has been estimated to be hundreds to tens of thousands of years depending on feedstock and pyrolysis conditions (Zimmerman, 2010). This leads to carbon storage in the soil and its removal from the atmosphere (Lehmann, 2007). In addition, modest additions of biochar to soil have been found to reduce emissions of greenhouse gases from cultivated soils, for example, reducing N_2O emissions by up to 80% and completely suppressing methane emissions (Lehmann *et al.*, 2006; Rondon *et al.*, 2007; Yanai *et al.*, 2007). When contemplated as a part of this 4-part "Charcoal Vision" involving renewable energy generation, waste treatment, soil fertility improvement, and carbon sequestration (Laird, 2008), charcoal has earned a new moniker: BIOCHAR.

Currently, biochar is hardly utilized in modern agriculture, and its agronomic value in terms of crop response and soil health benefits has yet to be quantified. Impediments to the adoption of biochar use in modern agriculture are many, and include the great variability in biochar characteristics as a

function of feedstock and pyrolysis conditions, particularly pyrolysis highest treatment temperature (HTT). Biochars produced at relatively low temperatures (below about 500°C) have substantially different characteristics than those produced at high temperatures (above about 550°C). Compared with high HTT biochars, low HTT biochars have lower pH values (neutral to mildly alkaline), lower ash contents, lower specific surface areas (SSA), and higher cation exchange capacities (CEC) per unit surface area. These characteristics can influence biochar suitability as a soil amendment in yet unknown ways, as well as its stability in the environment, which can affect its utility as a long term carbon sink.

'The Biochar Effect' on plant growth

Various publications report a generally positive effect of biochar soil amendment on field crops and trees grown under greenhouse and commercial conditions. Early studies reported that charcoal added to soil increased the yield of moong, soybean and pea (Iswaran *et al.*, 1980) and of soybean (Kishimoto and Sugiura, 1985). Shoot and root biomass of birch and pine were greater in charcoal-amended soil (Wardle *et al.*, 1998). Similarly, five years following the soil application of charcoal, biomass production of sugi trees (*Cryptomeria japonica*) was substantially increased (Kishimoto and Sugiura, 1985). A single application of 20 t ha^{-1} biochar to a Colombian savanna soil resulted in an increase in maize yield by 28 to 140% as compared with the unamended control in the 2nd to 4th years after application (Major *et al.*, 2010). With addition of biochar (at 90 g kg^{-1}) to a tropical, low-fertility Ferralsol, the proportion of N fixed by bean plants (*Phaseolus vulgaris*) increased from 50% (without biochar) to 72%, and biomass production and bean yield were significantly improved (Rondon *et al.*, 2007). On the same type of soil, total N recovery in soil, crop residues, and grains was significantly higher on compost (16.5%), charcoal (18.1%), and charcoal plus compost treatments (17.4%) in comparison to mineral-fertilized plots (10.9%) (Steiner *et al.*, 2008b). Biochar soil application resulted in higher upland rice (*Oryza sativa*) grain yields at sites in northern Laos with low P availability, and improved the response to N and NP chemical fertilizer treatments (Asai *et al.*, 2009). Large volume applications of biochar (30 and 60 t ha^{-1}) in the Mediterranean basin increased durum wheat (*Triticum durum*) biomass and yield by up to 30%, an effect which was sustained for two consecutive seasons (Vaccari *et al.*, 2011). Overall, these results demonstrate the potential of biochar application to improve plant productivity.

The means by which biochar improves crop response can be attributed to direct effects via biochar-supplied nutrients (Silber *et al.*, 2010), and to several other indirect effects, including: increased nutrient retention (Chan *et al.*, 2007, 2008; Chan and Xu, 2009); improvements in soil pH (Yamato *et al.*, 2006; Steiner *et al.*, 2007; Novak *et al.*, 2009); increased soil cation exchange capacity (Cheng *et al.*, 2006; Yamato *et al.*, 2006; Novak *et al.*, 2009); effects on P and S transformations and turnover (Pietikainen *et al.*, 2000; DeLuca *et al.*, 2009); neutralization of phytotoxic compounds in the soil (Wardle *et al.*, 1998); improved soil physical properties including water retention (Iswaran *et al.*, 1980; Ballestero and Douglas, 1996; Glaser *et al.*, 2002; Chan *et al.*, 2008; Laird *et al.*, 2009; Novak *et al.*, 2009); promotion of mycorrhizal fungi (Yamato *et al.*, 2006; Rondon *et al.*, 2007; Warnock *et al.*, 2007); and alteration of soil microbial populations and functions (Pietikainen *et al.*, 2000; Steiner *et al.*, 2008a; Graber *et al.*, 2010; Kolton *et al.*, 2011). Many of these effects are interrelated and may act synergistically to improve crop performance. Specific mechanisms underlying the contribution of biochar to plant response are poorly understood. Regional conditions including climate, soil chemistry and soil condition all influence biochar agronomic benefits. In addition, dissimilar biomass feedstocks and pyrolysis conditions create biochars with different physical and chemical properties (Keiluweit *et al.*, 2010), resulting in different impacts on plant response (Chan *et al.*, 2007, 2008).

Given that the biochar-soil-plant-water-environment is highly complex, it is difficult to isolate those factors which actually play an instrumental role in the 'Biochar Effect'. To reduce the number of potential factors involved, Graber *et al.* (2010) tested whether biochar addition could impact plant growth when nutritional and soil physical aspects of biochar amendment were eliminated. This was achieved by examining the impact of a nutrient-poor, wood-derived biochar on tomato

(*Solanum lycopersicum*) and pepper (*Capsicum annuum*) development in a commercial coconut fiber:tuff soilless mixture under an optimal fertigation (fertilization plus irrigation) regime in a greenhouse. They reported an increase in several plant growth parameters (height, leaf area, canopy) for both pepper and tomato plants under biochar-treatment (1–5% w:w). The positive impacts of biochar on pepper and tomato plant response were not due to either direct or indirect effects on plant nutrition (no effect of biochar amendment on leaf nutrient content), or to improvements in water holding capacity of the soilless mixture (no difference due to biochar addition). As such, they concluded that biochar-induced plant growth stimulation goes beyond obvious contributions to plant nutrition and improved soil physical and chemical properties. They proposed two related hypotheses to explain the improved plant performance under biochar treatment: (i) biochar addition caused a shift in microbial populations towards beneficial plant growth promoting rhizobacteria (PGPR) or fungi (PGPF) as a result of either chemical or physical attributes of the biochar; or (ii) low doses of biochar-borne chemicals, many of which are phytotoxic or biocidal at high concentrations, stimulated plant growth at low doses (hormesis).

Biochar stimulation of beneficial soil microflora

Evidence is mounting that biochar in soil has significant effects on soil micro-organisms, as reviewed recently by Lehmann *et al.* (2011). In the majority of the studies assessed in that review, microbial biomass increased in biochar-amended soils. Biochar addition also caused significant changes in microbial community composition and enzyme activities in both bulk soil and the rhizosphere. For instance, biochar amendment was generally characterized by an increase in the relative abundance of members of the Actinobacteria and Bacteriodetes phyla (Jesus *et al.*, 2009; O'Neill *et al.*, 2009; Khodadad *et al.*, 2011; Kolton *et al.*, 2011). While little is understood regarding the mechanisms by which biochar affects microbial abundance and community structure, it is well-known that soil micro-organisms can have a tremendous impact on plant productivity. Therefore, biochar-induced changes in soil micro-organisms may certainly play a role in 'The Biochar Effect' (Graber *et al.*, 2010).

A major family of soil micro-organisms that is well known for its positive impact on plant productivity is arbuscular-mycorrhizal (AM) fungi. AM fungi are obligate symbiotic soil fungi which colonize the roots of vascular plants (Mosse, 1957). A conservative estimate suggests that 80% of terrestrial land plants are potential hosts to these fungi (Bonfante-Fasolo, 1987). This symbiosis typically results in enhanced host vigor, most frequently demonstrated in increased uptake of immobile nutrients, principally phosphorus, from the soil (Harrison, 1999). Among the many other benefits conferred by this symbiosis, resistance to drought and pest tolerance are among the most notable (Nelsen and Safir, 1982). Modulation of pathogenesis related (PR) proteins and phytohormones (especially gibberellins) in the host plant are known to play a role in AM fungal–host symbiosis (Shaul *et al.*, 1999; Shaul-Keinan *et al.*, 2002). Addition of biochar to soil often results in significant augmentation of mycorrhizal fungi-plant symbiotic interactions (Warnock *et al.*, 2007). For instance, mycorrhizal colonization of wheat roots and wheat grain yield increased significantly under biochar application together with mineral fertilizer. Application of biochar and fertilizer also increased mycorrhizal colonization in clover bioassay plants, and it was concluded that biochar provided suitable conditions for mycorrhizal fungi to colonize plant roots (Solaiman *et al.*, 2010). Warnock *et al.* (2007) summarized four mechanisms by which biochar may influence mycorrhizal abundance and/or functioning: (i) alteration of soil physico-chemical properties; (ii) indirect effects on mycorrhizae through effects on other soil microbes; (iii) plant-fungus signaling interference and detoxification of toxic chemicals on biochar; and (iv) provision of refuge from fungal grazers (Warnock *et al.*, 2007). Such mechanisms may also affect other soil dwelling fungi including plant pathogens.

Beyond the well-known role of arbuscular mycorrhizal (AM) fungi in promoting plant growth, it is known that rhizosphere microorganisms in general, and selected strains belonging to the genera *Pseudomonas*, *Bacillus*, and *Trichoderma* in particular, can improve plant growth in many cropping systems. For instance, increased growth was triggered by species of *Trichoderma* in tomato, tobacco and radish (Windham *et al.*, 1986), and by species of *Bacillus* (Kloepper *et al.*, 2004) and

Pseudomonas (Mercado-Blanco and Bakker, 2007) in several crops. As yet, few studies have examined the proliferation of plant growth promoting rhizobacteria/fungi (PGPR/F) in biochar-amended soils. One notable work cultured rhizosphere and bulk soil from mature pepper plants whose growth was enhanced by biochar additions (Graber *et al*., 2010). Of the 20 distinct isolates obtained, phylogenetic characterization based on partial 16S rRNA gene analysis revealed that 8 of the isolates shared high sequence identity (98% or more) with *Pseudomonas*, *Mesorhizobium*, *Brevibacillus*, and *Bacillus* strains known for their ability to act as plant growth promoting agents (Graber *et al*., 2010). While this observation, the first of its kind, does not definitively demonstrate a role for biochar-stimulated bacteria in plant growth promotion, it does suggest an important future research direction that may help elucidate the "Biochar Effect". Many PGPR/F organisms are known to promote plant health in addition to plant growth, either by directly controlling plant pathogens or by potentiating plant systemic resistance responses against diseases and pests (Koike *et al*., 2001; Srinath *et al*., 2003; Gravel *et al*., 2007; Kaewchai *et al*., 2009). The impact of biochar on plant disease is explored in the following section.

Biochar effect on plant disease

Very few studies have addressed the potential for biochar soil amendment to impact plant resistance to disease pathogens. Regarding soil pathogens, Matsubara *et al*. (2002), while principally concerned with the effect of AM fungal inoculations on asparagus tolerance to fusarium root rot, incidentally demonstrated that charcoal amendments had a suppressive effect on the soil borne pathogen *Fusarium* sp. They found that charcoal produced from coconut fiber suppressed fusarium crown and root rot, and increased AM colonization of asparagus seedlings. A more recent study that supports these earlier findings reported that biochar made from ground hardwood added to asparagus field soil resulted in reductions in root lesions caused by *Fusarium oxysporum* f. sp. *asparagi* and *F. proliferatum* compared with a non-amended control (Elmer and Pignatello, 2011). Moreover, biochar amendments improved AM colonization of asparagus roots, contributing to suppression of the diseases even after the addition of allelopathic agents known to reduce AM colonization in asparagus (Elmer and Pignatello, 2011). These results reinforce the hypothesis that biochar may help ward off allelopathic effects via adsorption and detoxification of allelopathic agents, a phenomenon earlier noted by Wardle *et al*. (1998).

Besides detoxification of chemical agents, biochar suppression of soil pathogens may stem from several mechanisms, including: (i) providing nutrients and improving nutrient solubilization and uptake, which helps enhance plant growth and resistance to stresses of pathogenic soil micro-organisms; (ii) stimulation of microbes which provide direct protection against soil pathogens via antibiosis, competition, or parasitism; (iii) biochar-associated organic compounds may suppress sensitive components of the soil microbiota and result in proliferation of resistant microbial communities; (iv) biochar may induce systemic plant defense mechanisms, with elicitors being either/both biochar-borne chemicals or biochar-induced micro-organisms. The mechanisms by which biochar supplies nutrients and improves nutrient availability have been discussed in detail in recent reviews and studies (Chan and Xu, 2009; DeLuca *et al*., 2009; Atkinson *et al*., 2010; Silber *et al*., 2010), and will not be further addressed here.

Biochar is initially sterile and does not have an indigenous population of microorganisms that can potentiate disease suppression. Yet, biochar does influence microbial populations and communities as discussed above, and these changes may include an increase in beneficial microorganisms that directly protect against soil pathogens by producing antibiotics, by out-competing the pathogens, or by grazing on the pathogens. In addition, chemical compounds in the residual tars that are added to the soil with the biochar may have direct toxic effects on soil pathogens. For instance, Graber *et al*. (2010) identified a number of biochar compounds that are known to adversely affect microbial growth and survival. These include ethylene glycol and propylene glycol, hydroxy-propionic and butyric acids, benzoic acid and o-cresol, quinones (recorsinol and hydroquinone), and 2-phenoxyethanol. Low levels of these toxic compounds could suppress sensitive components of the soil microbiota thereby resulting in proliferation of resistant microbial communities. A possible indica-

tion of this was the identification of an isolate with 100% 16S rRNA gene sequence identity to the nitrophenol-degrader *Nocardioides nitrophenolicus* in biochar-amended soil (Graber *et al.*, 2010). Microorganisms which excel at degrading toxic organic contaminants generally are more resistant to a variety of toxic organic compounds. Also, antibiotic and volatile organic compound producers are often resistant to a multitude of antibiotics (Nodwell, 2007; Laskaris *et al.*, 2010). Antibiotic producers (*Pseudomonas mendocina* and *P. aeruginosa* strains) were identified in biochar-amended soil (Graber *et al.*, 2010).

The possibility that biochar induces plant systemic resistance responses against disease microorganisms has been studied in several different systems involving foliar pathogens. The severity of diseases caused by necrotrophic (*Botrytis cinerea*) and biotrophic (*Oidiopsis sicula* (originally referred to according to its teleomorph name: *Leveillula taurica*)) foliar pathogens in pepper and tomato (Elad *et al.*, 2010) was significantly reduced in biochar-amended treatments. Reduced damage by broad mite (*Polyphagotarsonemus latus*) in biochar-amended pepper plants was also observed (Elad *et al.*, 2010). Biochar soil amendment moreover resulted in suppression of *Podosphaera aphanis*, *B. cinerea* and *Colletotrichum acutatum* on the leaves of strawberry plants (Meller Harel *et al.*, 2012a). The fact that the biochar location during all stages of plant development was spatially separate from the site of infection indicates there was no direct toxicity towards the causal agents, and points to an induced systemic response of the plant against the pathogens. Improved plant nutrition and water balance were eliminated as factors in the induced response (Graber *et al.*, 2010).

Induced resistance in plants, effective against a broad range of pathogens and parasites including fungi, bacteria, viruses and nematodes, is a physiological state of enhanced defensive capacity elicited by specific stimuli, whereby the plant's innate defenses are potentiated against subsequent challenges (Vallad and Goodman, 2004). Two forms of induced resistance defined in model plant systems are Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR). They can be differentiated by their regulatory pathways and the nature of the elicitors. SAR is associated with the production of PR proteins and mediated via a salicylic acid (SA)-dependent process. SAR usually develops subsequent to a localized hypersensitive reaction. ISR develops systemically in response to colonization of plant roots by PGPR and fungi (PGPF) (Van der Ent *et al.*, 2009). This type of resistance is mediated by a signaling pathway in which the phytohormones jasmonic acid and ethylene play key roles. ISR does not involve expression of PR proteins (Van Loon *et al.*, 1998). Both chemical elicitors and biological elicitors (virulent, avirulent, and nonpathogenic microorganisms) can trigger SAR (Vallad and Goodman, 2004). For example, *Trichoderma* spp. can release compounds that induce SAR much as they elicit ISR (Harman *et al.*, 2004). Chemical inducers of systemic resistance include the synthetic SA-analogues 2,6-dichloroisoniciotinic acid and acibenzolar-S-methyl (Iriti *et al.*, 2004; Perazzolli *et al.*, 2008), methyl jasmonate (Belhadj *et al.*, 2006), chitin (Rajkumar *et al.*, 2008a) and chitosan (Aziz *et al.*, 2006), laminarin (Trouvelot *et al.*, 2008), and β-aminobutyric acid (Hamiduzzaman *et al.*, 2005). Phosphate salts, silicon, amino acids, fatty acids, and cell wall fragments can also stimulate systemic resistance (Reuveni *et al.*, 1995; Walters *et al.*, 2005; Wiese *et al.*, 2005), as can environmental agents such as osmotic, moisture and proton stresses, mechanical wounding, and temperature extremes (Ayres, 1984; Wiese *et al.*, 2004).

Frequently, induced disease resistance is associated with an overall heightened capacity of the plant to induce cellular defense responses upon encountering stresses, i.e., "the primed state of the plant" (Ton and Maunch-Mani, 2003). Primed plants display faster and stronger activation of cellular defense responses after pathogen challenge as compared with non-primed plants (Conrath *et al.*, 2006), including earlier oxidative burst and stronger up-regulation of defense genes (Conrath *et al.*, 2002; Ahn *et al.*, 2007). While the physiological and molecular mechanisms underlying primed responses are widely unknown, priming has been observed to be an integral part of both SAR and ISR.

Molecular evidence for systemic induction of plant defenses via both SAR and ISR pathways by biochar was recently presented (Meller Harel *et al.*, 2012a; Meller Harel *et al.*, 2012b). Biochar addition to the potting medium of strawberry plants suppressed foliar diseases caused by fungi having

very different infection strategies: necrotrophic (grey mould, *Botrytis cinerea*), semi-biotrophic (anthracnose, *Colletotrichum acutatum*), and biotrophic (powdery mildew, *Podosphaera apahanis*). The relative expression of five defense-related genes (FaPR1, Faolp2, Fra a3, Falox, and FaWRKY1) in leaves was significantly increased by 1–3% biochar amendment to roots of plants grown in potting mix, indicating that biochar amendment triggered both salicylic acid and jasmonic acid/ethylene-induced gene expression in the leaves. Furthermore, plants were primed for gene expression upon infection by *B. cinerea* and by *P. aphanis*. Thus, changes induced by root exposure to biochar took place along both SAR and in ISR pathways, resulting in a broad spectrum controlling capacity.

The question arises, by what mechanism(s) does biochar induce SAR and ISR pathways? ISR develops systemically in response to colonization of plant roots by PGPR and PGPF (Van der Ent *et al.*, 2009). PGPRs or PGPFs in combination may also have a synergistic effect on both plant growth promotion and biocontrol, as shown, for example, for *Bacillus*, *Pseudomonas* and *Chryseobacterium* spp. in both tomato and pepper plants (Domenech *et al.*, 2006). Graber *et al.* (2010) isolated a number of bacteria with high 16S rRNA gene sequence identity to known biocontrol agents, induced resistance agents and growth promoters (15 out of 20 total isolates) from the root zone of biochar-amended pepper plants where promotion of plant growth and induction of systemic resistance against fungal foliar diseases occurred simultaneously (Elad *et al.*, 2010; Graber *et al.*, 2010). In a follow-up study, culture independent molecular fingerprinting (DGGE and T-RFLP) of 16S rRNA gene fragments showed a clear differentiation between the root-associated bacterial community structures of the biochar-amended and control pepper plants, with a significant increase in the relative abundance of members of the Bacterioidetes phylum in the biochar-amended samples (Kolton *et al.*, 2011). The Bacteroidetes-affiliated *Flavobacterium* was the genus most strongly induced by the biochar. Members of the *Flavobacterium* genus, which is widely distributed in nature, commonly possess an arsenal of extracellular enzymes such as proteinases and chitinases which enable them to degrade bacteria, fungi, insects and nematode constituents (Bernardet and Bowman, 2006). Furthermore, members of the *Flavobacterium* genus often produce secondary metabolites, including a wide range of antibiotics (Clark *et al.*, 2009), and some *Flavobacterium* isolates have been shown to have biocontrol capabilities. For instance, selected *Flavobacterium* isolates were highly antagonistic toward the soilborne fungal pathogens *Sclerotium rolfsii*, *Lasiodiplodia theobromae*, *Colletotrichum musae*, and *Phytophthora cactorum*, which can infect a range of agricultural and horticultural crops (Hebbar *et al.*, 1991; Alexander and Stewart, 2001; Gunasinghe and Karunaratne, 2009). In addition, some *Flavobacterium* strains were capable of eliciting a resistance response of plants to different diseases (An *et al.*, 2008; An *et al.*, 2009). Other hydrolytic enzyme-producing genera including *Chitinophaga* (Bacteroidetes) and *Cellvibrio* (Betaproteobacteria) were also induced in the rhizosphere of the biochar-amended pepper plants (Kolton *et al.*, 2011). Such biopolymer-degrading bacteria may mineralize chitin in the outer shells and cell walls of rhizosphere-associated arthropods and fungi (Kim and Jung, 2007), releasing chitin oligomers to the soil environment. Chitin oligomers are well known elicitors of ISR (Rajkumar *et al.*, 2008b). Interestingly, biochar-amendment was found to be antagonistic towards the *Pseudoxanthomonas* genus (Gammaproteobacteria) (Kolton *et al.*, 2011); several *Pseudoxanthomonas* species are known opportunistic plant pathogens which attack a diverse array of economically important crops (Thierry *et al.*, 2004).

SAR may be induced by various chemical agents in addition to induction by microorganisms. Biochars contain residual tars comprised of a complex mixture of dozens of individual organic compounds from a number of major chemical classes, including medium and long chain *n*-alkanoic acids, hydroxy and acetoxy acids, benzoic acids, short and medium chain diols and triols, phenols and polyphenols, amines, amides, and aliphatic hydrocarbons (Schnitzer *et al.*, 2007; Schnitzer *et al.*, 2008; Graber *et al.*, 2010). These compounds are present in the biochar at relatively low levels. Graber *et al.* (2010) hypothesized that plants in the biochar-amended soils could respond to the stress of low levels of phytotoxic compounds in the root zone via an induced resistance mechanism such as reported for *Arabidopsis thaliana* in the presence of low levels of (±)-catechin (Prithiviraj

et al., 2007). In that study, *A. thaliana* exhibited an inverted U-shaped growth response to (±)-catechin, which is phytotoxic at high concentrations. At low (±)-catechin concentrations, where growth was promoted, plant leaves inoculated with *Pseudomonas syringae* pv. *tomato*, a pathogen of *A. thaliana*, developed restricted lesions only at the site of inoculation, while the control plants exhibited widespread infection, indicating the development of systemic induced resistance in the face of low levels of phytotoxic compounds (Prithiviraj *et al.*, 2007).

It remains to be seen what types of biochar can induce resistance responses, considering the very big variability in physical and chemical properties that biochars exhibit, depending on original feedstock and pyrolysis conditions (Amonette and Joseph, 2009; Downie *et al.*, 2009; Krull *et al.*, 2009). In unpublished work from our group, the effect of biochars produced at two pyrolysis HTTs (350 and 450°C) from three biomass feedstocks (greenhouse waste, olive pomace, and Eucalyptus wood) on infection of tomato leaves by *B. cinerea* was tested. Tomato plants were grown under controlled greenhouse conditions under full fertigation in a potting mixture amended with the biochars at different rates (0, 0.5, 1, and 3% by weight). In most cases, the biochars induced resistance towards grey mould regardless the feedstock, pyrolysis HTT, grey mould assay (whole plant attached leaves vs. detached leaves), period of exposure to the biochar, and plant age (Table 1). Nevertheless, we expect that disease control efficacy will vary with other biochar production temperatures, biomass sources, plant growth systems, plant species and diseases. An example of resistance induced by greenhouse waste biochar towards grey mould infection in the canopy of tomato plants is presented in Figure 1.

Biochar interaction with soil pesticides

In a review paper dedicated to phytopathological aspects of biochar addition to soil, it would be remiss not to point out that biochar addition may negatively impact the efficacy of soil-applied pest products, including fungicides, insecticides, and herbicides, due to the high adsorption affinity and capacity that many biochars exhibit towards numerous organic compounds. Alternatively, the soluble organic component of biochar may form complexes with soil-applied herbicides, and thus enhance their downward transport out of the soil zone (Cabrera *et al.*, 2011). A progressive increase in adsorption with increasing biochar content was demonstrated for the fungicide pyrimethanil (Yu *et al.*, 2010). Strong adsorption of pesticides on applied biochar can result in pesticide inactivation (Graber *et al.*, 2011b; Nag *et al.*, 2011), such that greater pesticide amounts may be needed to obtain the same level of protection against pests, for example, as seen in a study of fumigant efficacy against nematodes in biochar-amended soil (Graber *et al.*, 2011b). Biochars having high surface areas (specific surface area; SSA) can be particularly challenging for pest control, since for many compounds, their adsorption strength is commonly much greater than that of low SSA biochars (Bornemann *et al.*, 2007; Chen and Chen, 2009; Wang *et al.*, 2010; Yang *et al.*, 2010). Biochar SSA generally increases with increasing pyrolysis temperature. When a soil was amended with 2% (52 t ha^{-1}) high SSA biochar (SSA of 242 $m^2 g^{-1}$), control of the weed Green foxtail (*Setaria viridis*) was significantly diminished, even when maximum herbicide (S-metolachlor and sulfentrazone) label rates were used (Graber *et al.*, 2011a). At 1% (26 t ha^{-1}) biochar however, the high herbicide rates were sufficient to control the weed growth. In soil amended with low SSA biochar (SSA of 3 $m^2 g^{-1}$), weed growth was well controlled even at low label rates of the herbicides. Other biochar attributes that may influence absorption ability include CEC, particularly for cationic substances, and oxidation status of biochar surface functional groups. While there is no "standard biochar application rate", the vast majority of reported field trials with biochar have been conducted at levels of biochar application ranging from 1 to 20 t ha^{-1} (Blackwell *et al.*, 2009). The potential challenges for pest control posed by biochar addition to soil (reduced pesticide efficacy and bioavailability; increased pesticide accumulation) have been recently reviewed (Kookana, 2010).

Future perspectives, constrains, problems

Clearly, more research into understanding the impacts of biochar soil amendments on plant growth and resistance to biotic (and abiotic) stresses is needed. Until now, most studies have focused

Table 1. Effect of adding biochars[a] produced from different feedstocks at HTTs of 350 and 450°C on the severity of tomato leaf grey mould.

Biomass source	Pyrolysis temperature (°C)	Assay method[b]	Plants age (days after planting)	Days after infection by *Botrytis cinerea*	Disease severity (±SE)[c] at biochar concentrations (%)			
					0	0.5	1	3
Greenhouse waste	350	AL	29	12	58 ± 7.8 a[d]	40 ± 2.2 b	35 ± 2.7 b	34 ± 4.0 b
Greenhouse waste	450	AL	72	14	50 ±13.6 a	nt	10 ± 1.7 c	17 ± 3.6 b
Olive pomice	350	DL	23	7	58 ± 6.5 a	34 ± 3.1 b	34 ± 3.0 b	32 ± 4.6 b
Olive pomice	450	DL	16	10	34 ± 1.7 a	nt	15 ± 1.5 b	11 ± 0.6 b
Olive pomice	450	AL	21	5	16 ± 3.1 a	nt	8 ± 2.6 b	13 ± 2.4 ab
Eucalyptus wood	350	DL	23	7	64 ± 5.7 a	50 ± 6.8 ab	32 ± 3.5 c	44 ± 5.5 b
Eucalyptus wood	350	AL	47	10	58 ± 6.8 a	25 ± 3.3 b	20 ± 4.2 b	27 ± 1.1 b

[a] The biochars were prepared from greenhouse waste (pepper plants collected at the end of the growing season), olive pomice residues from olive oil pressing, and eucalyptus chips in a slow pyrolysis unit. The biochars were ground into a powder of less than 1 mm particles. Each biochar powder was mixed with a coconut fiber:tuff (unsorted to 8 mm) (7:3 vol.:vol.) potting mixture. Plants of tomato cv. 1402 (Hazera Genetics, Ltd., Brurim M.P. Shikmim, Israel) were obtained from a commercial nursery (Hishtil, Ashkelon, Israel) at 40 to 50 days after seeding and transplanted into 3 L-pots containing the potting medium without or with biochar at 0.5 to 3% by weight. Plants were fertigated proportionally with drippers 2–3 times per day with 5:3:8 NPK fertilizer (irrigation water was planned to have total N, P and K concentrations of 120, 30 and 150 mg L^{-1}, respectively; EC 2.2 dS/m), allowing for 25–50% drainage. Plants were maintained at 23 to 27ºC in a pest- and disease-free greenhouse 16–72 days and then transferred to a controlled temperature chamber where the disease was allowed to develop under high humidity conditions following inoculation of intact or detached leaves.

[b] AL, Intact leaves attached to the plant; DL, leaves detached from the plant. Both types of assays were carried out in a humidity chamber at 20±1°C, 97±3% RH, and 1020 lux light intensity.

[c] *Botrytis cinerea* (isolate BcI16; [Swartzberg *et al*., 2008]) culture and conidia separation was carried out according to (Elad *et al*., 2010). The conidia suspension was then filtered through cheesecloth. The concentration of conidia was determined using a haemocytometer and a light microscope, and adjusted to 5×10^5 cells mL^{-1}. To facilitate germination of *B. cinerea* conidia and subsequent leaf infection 0.1% glucose was added to the final conidial suspension together with 0.1% KH_2PO_4 (Elad and Yunis, 1993; Cole *et al*., 1996). For detached leaves assays, five leaves/plant from at least five plants were each inoculated with a 10 µL drop of a 5×10^5 conidia mL^{-1} suspension. The severity of the resulting necrotic lesion on each leaf was determined according a scale of 0 to 100% (Guetsky *et al*., 2001). Whole plants were sprayed with the conidia suspension (2 mm $plant^{-1}$) and incubated in a polyethylene bag during the entire period of disease development. Five to six plants were used for each treatment.

[d] Data in each row labeled by a common letter are not significantly different according to Fisher's protected LSD test. Nt = not tested.

on direct or indirect effects of biochar on plant nutrition and soil structure to explain the improved plant productivity under biochar addition. However, having demonstrated a positive impact of biochar amendment on plant growth stimulation (pepper, tomato) and defense responses (pepper, tomato and strawberry) under optimal fertigation regimes in a well-structured soilless growth medium, where neither direct nor indirect effects on nutrition, soil structure or water retention played a role, it is evident that alternative mechanisms must have made major contributions to the observed stimulatory effect of the biochars. Alternatives are abundant and require testing: (i) biochar stimulates the development of beneficial microorganisms which promote plant growth and induce resistance to pathogens; (ii) biochar-borne chemicals directly elicit positive plant responses; (iii) biochar neutralizes pathogen-produced plant toxins; (iv) biochar adsorption or alteration of root exudates impacts the rhizosphere microbial community; and more. Studies to test the isolated microbes and biochar-borne chemicals for their potential activity in promoting plant growth and eliciting disease resistance in different systems are currently underway by our group.

Clear shifts in the root-associated microbial community structure of plants grown in biochar amended soil have been observed, characterized

Figure 1. Tomato plants infected by *Botrytis cinerea* conidia spray at ten days following infection. Plants were grown in a coconut fiber:tuff potting mixture without biochar (left) or amended with 1% (w:w) greenhouse waste biochar (GHW-350) produced at an HTT of 350°C (right). *B. cinerea* infection was made after three week-growth of the plants in a 23–27°C growth chamber with no pathogens.

by substantial induction of several chitin- and aromatic compound-degrading genera. It was suggested that physical and chemical factors (biochar-associated organic compounds) may be collectively responsible for the observed community shift, and that induced bacterial communities may be at least partially responsible for the induced growth and plant resistance phenomena observed. It should also be recalled that biochar-associated residual tar compounds could potentially have phytohormone-like activity, which may influence the Biochar Effect. This possibility also deserves investigation.

Future research is warranted to decipher the induced resistance pathways, as well as to examine the impact of biochar on plant priming against abiotic stresses. Improved plant resistance to stress is yet an additional benefit potentially associated with biochar application in soil, together with improved crop production, soil water retention, and soil tilth. A major unknown is the effect of biochar feedstock and pyrolysis conditions on the response of plants to stresses, and indeed, on growth promotion. Moreover, there is a need to address potential risks associated with biochar use, and to determine the best means of using biochar in agricultural systems. In the future, the development of agricultural markets for biochar products, including as a disease control agent, can help promote the adoption of biomass pyrolysis as an important tool in both mitigation of, and adaptation to, climate change.

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