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Evaluation of compost amendments for suppressiveness against Verticillium wilt of eggplant and study of mode of action using a novel Arabidopsis pathosystem

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Abstract

The induced resistance potential of eleven compost samples that originated from four different countries (Greece, France, Netherlands and Israel) and were manufactured from various raw materials, was evaluated in an *Arabidopsis thaliana–Verticillium dahliae* pathosystem under greenhouse conditions using a novel Plexiglas chamber. Five out of eleven composts tested showed significant disease suppressiveness compared to the control treatment; three composts exhibited disease severity equal to the control, while in the other three composts, disease severity was higher than the control treatment. Two of the tested composts that showed strong or medium suppressiveness were further evaluated under field conditions against Verticillium wilt of eggplant. Neither of them significantly reduced disease severity or resulted in higher fruit yield in a semi-commercial field test although they could induce a systemic resistance response in the greenhouse. However, as a consequence of a growth-promoting effect, one of the compost samples tested in the field resulted in a significant yield increase compared with the other.

Introduction

A number of reports in the literature refer to the potential of composts to suppress diseases initiated by soilborne pathogens, when applied as mulches to the soil surface, incorporated as amendments or as components of potting mixes (Hoitink and Boehm, 1999). Among them, management of Pythium and Phytophthora root rots, (Lumsden et al., 1983; Schüler et al., 1989) and Rhizoctonia damping-off (Nelson et al., 1983; Tuitert et al., 1988; Paplomatas et al., 2004) are examples of successful biological control through the use of compost-amended substrates. In many cases, compost-mediated suppressiveness is fortified by the addition of various biocontrol agents like *Trichoderma* spp. or *Bacillus subtilis*, during or immediately after peak heating in order to outgrow other mesophilic microbial colonizers (Kwok et al., 1987; Phae et al., 1990; Róckeboer et al., 1988).

It has been shown that usually more than one biocontrol agent is involved in compost suppressiveness against plant pathogens (Trillas-Gay et al., 1986; Kwok et al., 1987). Among the novel mechanisms of compost-driven disease suppression, induced resistance has been suggested by several researchers; Tränker (1992) observed that disease severity of wheat and barley powdery mildew was reduced in compost-amended than unamended soils, while Zhang et al. (1996) found that composted spruce and pine bark-amended soil or substrate mixes induced systemic acquired resistance (SAR) in cucumber against Pythium root rot and anthracnose caused by *Colletotrichum orbiculare*. Compost water extracts applied as topical sprays reduced symptoms of bacterial speck and population size of *Pseudomonas syringae* pv. *maculicola* in *Arabidopsis thaliana* plants grown in peat mix but not in the compost mix (Zhang et al., 1998). In this study, it was reported that β-1,3-glucanase activity was enhanced in cucumber plants grown in compost mix after inoculation by *C. orbiculare* and β-D-glucuronidase (GUS) activity driven by a β-1,3-glucanase gene promoter was similarly increased in transgenic Arabidopsis plants. Since GUS activity in the latter case was induced with compost water extract sprays or salicylic acid (SA) in non-inoculated plants, it was suggested that compost-induced disease suppression most likely involved the potentiation rather than the activation of resistance and in this respect compost-induced SAR could differ from SAR induced by pathogens, SA or compost water extract (Zhang et al., 1998).

Verticillium wilt is a devastating disease of a wide range of herbaceous and wood plant hosts, initiated by the soilborne fungus *Verticillium dahliae*. The disease causes very serious economic losses to a large number of crops, since there are no chemical treatments to control it (except for soil fumigation that is only applied to high-value crops usually in greenhouse cultivations). Thus, management strategies are focused on preventive measures; among those is the use of resistant hosts or attempts at biological control practices (Tjamos, 1989). Therefore, the use of compost soil amendments should be evaluated as an alternative to control *V. dahliae*, especially considering the upcoming banning of the use of methyl bromide, the most effective soil fumigant available (Ajwa et al., 2003).

In view of all the above, the objectives of the present study were (i) to determine whether composts can induce resistance in the *A. thaliana*–*V. dahliae* pathosystem and (ii) to evaluate compost suppressiveness against Verticillium wilt under field conditions. *Arabidopsis thaliana* was selected as a model plant because its genome has been fully characterized; this will facilitate future investigations of induced resistance mechanisms at the molecular level.

Materials and methods

Greenhouse experiments

A bioassay chamber made of Plexiglas was devised to evaluate the potential of various composts to induce resistance in Arabidopsis against *V. dahliae*. Each plexiglas unit was composed of two separate compartments, the upper (4 × 60 × 2 cm [width–length–height]) and the lower (4 × 60 × 5 cm); the touching sides were vertically incised at ten places, at a distance of 6 cm (Figure 1). The front part of each compartment was removable to facilitate placing of the plants. The roots of 10, 2-week old *A. thaliana* (ecotype Columbia) plants were placed vertically through incisions into the two opposing compartments. The roots of the upper part were covered by peat (Finnpeat, Kekkilä, Finland), while the roots of the lower compartment were covered by compost-peat mix (20:80 v/v). In the control treatment, the lower part was filled with peat only. The type and origin of the compost samples tested are presented in (Table 1). To ensure that all plants received the same level of nutrients, supplementary fertilization was added to each treatment after each compost sample was chemically analyzed for nutrient content. The appropriate amount of fertilizers was also added to the unamended control. Thus, any possible growth effect would be due to compost addition.

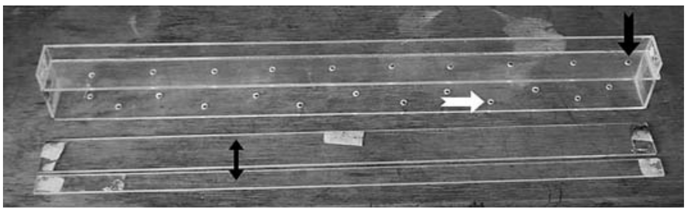

Figure 1. A novel plexiglas chamber to test induced resistance in *Arabidopsis thaliana* by various compost amendments. The black arrow denotes the incisions through which the Arabidopsis root system was separated into two parts, the lower (compost application) and the upper (pathogen inoculation). The white arrow indicates the drainage holes while the double-headed arrow shows the removable side parts that seal the two compartments with the aid of sticky tape.

Table 1. Origin, ingredients and composting details of composts tested for induced resistance in greenhouse pathogenicity tests against Verticillium wilt on *Arabidopsis thaliana*

Country of origin	Code name	Ingredients and composting details
France	CO2	Horse manure and green wastes 20:80 (green wastes composed of wheat and corn straws + conifer bark) Composted 4.5 months, matured 6 months
	CO4	Urban biowastes
	CO14	Grape marc and green wastes
	CO16	Woody wastes and poultry manure
	CO17	Woodcut, plants and horse manure, 10 months old
Greece	GR3	Spent mushroom composts: wheat straw, chicken manure, gypsum 100:70:10. Weathered for 6 months after harvesting of mushrooms
	GR5	Leonardite + urea + phosphate + catalyst 900:60:2020 Composted for 2–3 months in incubator. May be in pelleted form
	GR6	Horse manure and wood chips in a windrow tunnel
The Netherlands	2.01Ba	Wood chips (72%), manure (13.8%, 3 kinds of manure), clay (5%)
	2.01S	Wood chips (82%), manure (7.8% of 3 kinds of manure), clay (5%)
Israel	ISBS	Biosolids

Pathogen inoculum was produced by growing *V. dahliae* in sucrose sodium nitrate (SSN) liquid medium (Sinha and Wood, 1968) for 5 days at 22 °C and 120 rpm in a rotary shaker. One week after transplanting, the upper part of the roots was challenged by the pathogen by root drenching with 1 ml of a filtrated conidial suspension of *V. dahliae* (5×10^6 spores ml^{-1}). For the bioassay, Plexiglas chambers were arranged in a complete block design with three independent blocks. Each block included 12 treatments (11 different composts and one peat control), with treatments completely randomized within each block. For each treatment (plexiglas chamber), 10 plants (replications) were inoculated. Thus, the whole experiment included a total of 360 Arabidopsis plants (12 treatments $\times$ 10 replications $\times$ 3 blocks). All plants were kept on a greenhouse bench at 23 ± 2 °C and a 14 h photoperiod. The first wilting symptoms started 7 days after *V. dahliae* inoculation. Symptoms were recorded for about 2 weeks. Disease severity at each observation was calculated by the percent of leaves that showed wilting to the total number of leaves of each Arabidopsis plant. Subsequently, disease ratings were plotted over time to generate disease progress curves. The area under the disease progress curve (AUDPC) was calculated by the trapezoidal integration method (Campbell and Madden, 1990). Disease was expressed as a percentage of the maximum possible area for the whole period of the experiment (Korolev et al., 2001).

In order to evaluate the potential of each of the composts to induce resistance in *A. thaliana* against *V. dahliae*, statistical analysis of variance (ANOVA) of the disease severity for each treatment was performed. The results of the multivariate analysis of the data, when a significant ($P \leq 0.05$) *F*-test was obtained for treatments, were subjected to means separation by Duncan's multiple range test.

Field experiments

Based on disease suppressiveness evaluated in greenhouse bioassays and availability, selected composts were tested for their performance under semi-commercial field conditions. As a bioassay system, Verticillium wilt of eggplant was selected. Inoculum was prepared according to the following procedure. One isolate of *V. dahliae* that had originated from an infected eggplant was cultivated in liquid SSN medium for 4 weeks. In order to obtain microsclerotia, flasks were incubated under continuous shaking at 120 rpm and 22 °C in the dark. After growth, suspensions were homogenized using an omni mixer and then filtered through a mesh to hold microsclerotia over 70 μm diam., shown to be the most virulent

(Hawke and Lazarovits, 1994). Dried microsclerotia were kept at 4 °C until needed. Microsclerotia were then suspended in water to give the appropriate concentration after mixing with soil and incorporated into the composts at 50 propagules per g soil; infested composts were worked into the furrow to achieve a ratio of 20:80 (v/v) compost: soil. Treatments included two compost samples (GR3 and GR5) and an unamended control. The field experiment was performed in a complete block design. In each block, there were three treatments (two composts and one control) with 18 replications (plants) per treatment in two separate blocks. Eggplant seeds (cv. Black Beauty) were planted in trays with greenhouse soil mix and seedlings at the second true-leaf stage were transplanted in the field immediately after the incorporation of the inoculum. For the unamended control, inoculum (microsclerotia) was mixed with field soil alone. A total of 108 eggplants (2 blocks × 3 treatments × 18 plants) were planted in the experimental plot.

To ensure semi-commercial conditions, automatic drip irrigation lines were used for watering the whole field plot. Moreover, plants were spaced as in commercial fields, i.e. 30 × 30 cm (on and between rows), leaving a border line of 25 cm around each plot to minimize the effect on the experimental plants. Foliar symptoms of the disease were recorded at several intervals after inoculation. Disease was estimated with an infection rate that was calculated as the percent of leaf area with symptoms compared with the total leaf area of each plant. Subsequently, AUDPC values were calculated for each treatment by the trapezoidal integration method (Campbell and Madden, 1990). Disease was expressed as a percentage of the maximum possible area for the whole period (101 days after planting) of the experiment (Korolev et al., 2001). Except for disease suppression, yield was also measured to evaluate the growth promotion effects of the tested composts. Eggplant fruits from each plant were collected at two harvest times (approximately 2 and 3 months after transplanting) and fresh weight measured.

Data of disease severity and fruit yield for each treatment were statistically analyzed by ANOVA. When a significant ($P \leq 0.05$) F-test was obtained for treatments, data were subjected to means separation by Duncan's multiple range test.

Results

Induced resistance by composts

The capability of various compost samples to induce resistance in an *Arabidopsis–V. dahliae* model system was evaluated in greenhouse tests by using a novel bioassay chamber. Among the various composts, disease severity ranged between 28.0 and 260.8% compared with the unamended

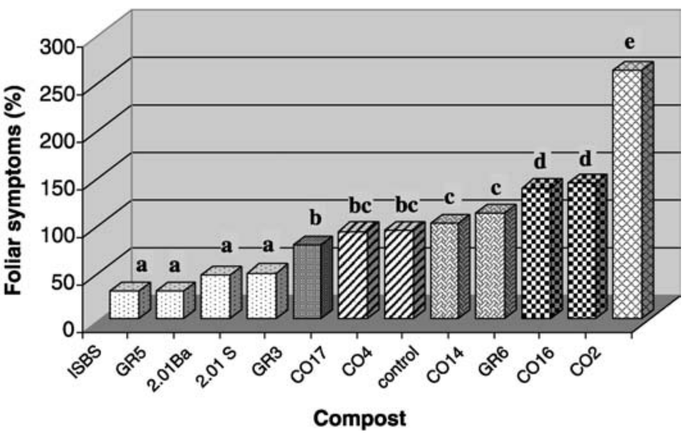

Figure 2. Effects of various compost samples on induction of resistance against Verticillium wilt of *Arabidopsis thaliana* plants, grown in compost amended soil and challenged with a conidial suspension of *Verticillium dahliae*. Disease severity was measured as foliar symptoms and expressed as percent of the unamended control. AUDPC values calculated for each treatment were subjected to ANOVA and means were separated by Duncan's multiple range test. Columns followed by the same letter are not significantly different at $P \leq 0.05$.

control (Figure 2). Five composts (ISBS, GR5, 2.01Ba, 2.01S and GR3) showed foliar symptoms that were significantly less than the control, three composts did not differ statistically from the control, while in three composts disease severity was increased compared with the control. Compared to control plants showing 100% disease severity, composts GR6, CO16 and CO2 were 137.00, 141.95 and 260.76% more severely infected. Thus, from the eleven different composts tested, SAR was induced by five of them while three others were conducive causing significant increases of disease severity compared with the unamended control (Figure 2). Compost GR5 had a pronounced growth promoting effect on Arabidopsis. Plants grown in GR5 amended soil showed at least three-fold foliar growth compared to the other compost amended treatments or the untreated peat control (Figures 3a, b).

Suppressiveness of composts under field conditions

Two Greek composts that showed strong (GR5) and medium (GR3) induced resistance properties in the greenhouse bioassays were selected for evaluation of their suppressive performance against Verticillium wilt of eggplant under field conditions. For both composts, disease severity measured as foliar wilt symptoms was not significantly reduced compared with the unamended control (Table 2). While disease severity in the control treatment reached 41.4%, for GR5 and GR3 it was 37.8 and 40.1%, respectively. Yield for both compost samples tested (790 for GR5 and 430 g per plant for GR3) was not significantly different from the unamended control (660 g per plant). However, eggplants grown in the GR5 amended treatment had a mean yield per plant that was significantly higher than the mean yield of plants grown in the GR3 amended plot (Table 2). Although the two compost samples tested in the field had a strong (GR5) or slight (GR5) potential to induce resistance in the *A. thaliana* model system tested, they were not able to significantly reduce Verticillium wilt foliar symptoms in a semi-commercial field situation. However, GR5 amendment resulted in a significant yield increase compared with GR3 treated eggplants, although not statistically different from the unamended control.

Discussion

The novel bioassay chamber proved to be very efficient in assessing induced resistance triggered by composts. It facilitated handling of Arabidopsis plantlets, allowed compartmentalization of the inducing factor (compost) from the challenger microorganism (*V. dahliae*) and was convenient in evaluating the progress of disease in the plants.

Although it has been demonstrated that composts can induce resistance, the mechanism(s) of this induction is still unknown. Among the variety of bacterial taxa that have been found to colonize compost-amended substrates, strains capable of inducing resistance in plants have been described (Han et al., 1998; Krause et al., 2003). Moreover, a

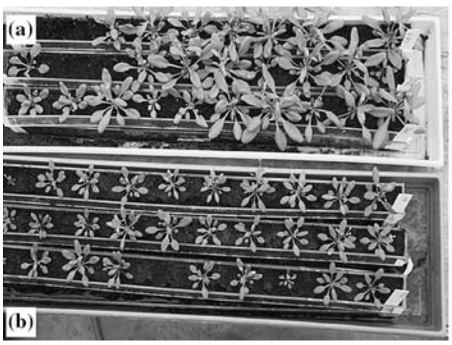

Figure 3. Arabidopsis thaliana plants grown in 20% (v/v) compost:peat mix and challenged with a conidial suspension of *Verticillium dahliae* in greenhouse bioassays using a novel plexiglas chamber. Compost GR5 had a pronounced growth effect on plants (a), while severely infected control plants showed a bronze discolouration of the leaves (b). Left (a + b): 14 days after *Verticillium dahliae* inoculation. Right (a + b): 24 days after *Verticillium dahliae* inoculation.

Table 2. Percent disease and yield of eggplants treated with selected composts and infested with *Verticillium dahliae* under semi-commercial field conditions

Treatment	% Disease	Yield (g/plant)
GR5	37.8 a	790 b
GR3	40.1 a	430 a
Control	41.4 a	660 b

Treatments with the same letter do not differ significantly from each other ($P \leq 0.05$).

certain population threshold of these particular strains must be reached before induction of systemic acquired resistance is evident (Raaijmakers et al., 1995). Our findings presented in this work showed that there were composts (ISBS, GR5) that strongly induced resistance (using the *V. dahliae–A. thaliana* system) but were not able to satisfactorily protect eggplants from Verticillium wilt; this was evident in preliminary greenhouse experiments (data not shown) and in experimental field results. On the other hand, there were compost samples (GR3) that showed similar plant protection for both pathogenicity assays in greenhouse experiments but not in a semi-commercial field situation. Finally, some compost samples (CO2) were not able to induce resistance in the Plexiglas chamber bioassay but had a strong disease suppressiveness of Verticillium wilt of eggplant under greenhouse conditions (data not shown).

The above compost behaviour could be attributed to the fact that in the induced resistance system compost samples were given the appropriate initial time to activate defensive mechanisms in Arabidopsis prior to challenging with the pathogen. In the eggplant pathogenicity tests, inoculum was incorporated into the compost just before its introduction into the soil; therefore the interaction of the pathogen was faster and thus limited the time of the compost to induce the defence mechanisms in eggplant. However, this different behaviour in the two pathosystems needs additional work and could be further investigated using DNA technology.

Based on the above, the different effects of the same compost in our induced resistance system and the greenhouse or field experiments could be explained up to a point. If a beneficial microorganism(s) population threshold is required (assuming that induction of resistance was of microbial origin), then in the Arabidopsis pathosystem the necessary time span required for such root colonization might be available. However, in the case of the field experiments where both compost and pathogen were simultaneously introduced into the system, disease was not suppressed as effectively as in the Arabidopsis pathosystem. Since this phenomenon could be an important component towards enhancing compost suppressive properties, further investigations of the mechanisms involved using population dynamics of microbial root colonization and molecular investigations of plant defence genes are necessary. Using an *A. thaliana–P. syringae* pv. *tomato* pathosystem, Vallad et al. (2003) were able to conclude that plants grown in soils amended with a paper mill residuals compost were more resistant to disease caused by the bacterium due to the induction of plant defences, similar to SAR.

Another surprising result in our induced resistance experiments was that three of the tested composts (GR6, CO16, CO2) significantly promoted Verticillium wilt in Arabidopsis compared with the unamended control (Figure 2). Although it has been documented that soil organic matter quality or concentration influences biocontrol agent activity (Hoitink and Boehm, 1999), in our case composts were selected on the basis of their maturity (data not shown). Moreover, there was no direct interaction of the compost microbial population with the challenging pathogen as in the case of compost incorporation into the infested soil substrates. At present, an explanation of disease enhancement by composts in the novel Plexiglas chamber is not possible. We can only speculate that a microbial component of compost suppressiveness should not be excluded, since these composts can be highly suppressive when they are in direct contact with the pathogen and plant roots.

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