

## Reproducibility of suppression of *Pythium* wilt of cucumber by compost

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There is increasing global interest in using compost to suppress soil-borne fungal and bacterial diseases and nematodes. We studied the reproducibility of compost suppressive capacity (SC) against *Pythium* wilt of cucumber using nine composts produced by the same composting plant in 2008 and 2009. A bioassay was set up in a greenhouse using cucumber inoculated with two strains of *Pythium*. The composts were used as 20% mixtures (v:v) of a basic steam-sterilized light *Sphagnum* peat and sand (3:1, v:v). Shoot height was measured weekly during the 5-week experiment. At harvest, the SC was calculated as the % difference in shoot dry weight (DW) between non-inoculated and inoculated cucumbers. The SC was not affected by year of production (2008 or 2009), indicating reproducibility of SC when the raw materials and the composting method are not changed. Differences in shoot height were not as pronounced as those for shoot DW. The results were encouraging, but further studies are still needed for producing compost with guaranteed suppressiveness properties.

*Key words:* Commercial composts, suppressive capacity, *Pythium* wilt, bioassay

### Introduction

When used in agriculture, horticulture or landscaping compost contributes to soil fertility, structure, porosity, organic matter, water holding capacity and disease suppression (Itävaara et al. 1997). The subject of disease suppression by composts represents a recently established alternative use of compost. The interest in this has increased due to concern over pesticide use, increasing incidences of pesticide resistance and paucity of chemical control compounds and disease resistant plant varieties. Hoitink et al. (1975) first suggested inclusion of compost in growing media to suppress soil-borne plant pathogens. Subsequently, the phenomenon of disease suppressiveness of composts has been addressed extensively in several reviews, for example by Hoitink and Fahy (1986), Hoitink and Boehm (1999), Noble and Coventry (2005) and Raviv (2008, 2009). The capacity of composts to suppress plant diseases is clearly linked with their degree of maturity (Kuter et al. 1988, Hadar and Gorecki 1991), although excessively stabilized composts may lose this ability (Hoitink and Grebus 1997).

Use of compost has suppressed globally important soil-borne pathogens such as *Pythium* Pringsh. spp. (Erhart et al. 1999), *Phytophthora* de Bary spp. (Hoitink and Boehm 1999), *Fusarium* Link spp. (Suárez-Estrella et al. 2007) and *Rhizoctonia* DC spp. (Nakasaka et al. 1998). There are examples also of inhibition of disease-causing bacteria (Schönfeld et al. 2003) and nematodes (Oka and Yermiyahu 2004) by using composts. Different mechanisms have been suggested to explain the disease suppression phenomenon (reviewed by Hadar and Papadopoulou 2012). These include physical and chemical mechanisms, like competition for nutrients and effects of humic and fulvic acids, or biological mechanisms, such as parasitism, antibiosis and systemic induced resistance caused by a consortium of compost microorganisms.

Species of *Pythium* cause damping-off and wilting in a broad range of plant species. Promising control of *Pythium* diseases using composts or compost water extracts has been reported in several studies. Pascual et al. (2002) noticed that the addition to soil of whole composts and their humic fractions reduced the effect of the pathogen on pea (*Pisum sativum* L.) plants. The greatest pathogen suppression was achieved with the chemical pesticides, but this also caused a significant decrease in the number of non-target bacteria and fungi and on beneficial soil microorganisms such as *Trichoderma* Persoon and *Pseudomonas* Migula. Nine out of seventeen composts from organic household waste were mildly suppressive to *Pythium ultimum* Trow in a study of Erhart et al. (1999). A bark compost also studied by them was even strongly suppressive. Hunter et al. (2006) found that sequences of Basidiomycete yeast genera and sequences highly similar to those of *Cryptococcus* Vuill. increased in their relative abundance in suppressive samples studied in a cress bioassay. The bacteria isolated, *Bacillus subtilis* (Ehrenb.)

Cohn and *B. thuringiensis* Berliner, acted antagonistically on the mycelial growth of *P. aphanidermatum* (Edson) Fitzp. in a study performed on tomato (*Solanum lycopersicum* L.) (Ben Jenana et al. 2009). Chen et al. (1988) studied suppressiveness of bark composts against damping-off caused by *Pythium ultimum*. They concluded that co-existence of large populations of mesophilic microorganisms, substantial microbial activity, low concentrations of available nutrients, and a high degree of microbiostasis characterized container media suppressive to *Pythium* damping-off. McKellar and Nelson (2003) achieved results indicating that communities of compost-inhabiting microorganisms colonizing cotton (*Gossypium hirsutum* L.) seeds within the first few hours after sowing in a *Pythium*-suppressive compost play a major role in the suppression of *P. ultimum* sporangium germination, seed colonization and damping-off. Results further indicate that fatty acid metabolism by these seed-colonizing bacterial consortia can explain the *Pythium* suppression observed.

Suppressiveness of composts against soil-borne disease has been the subject of many studies for more than two decades, but the practical applications of the phenomenon in the growing media industry and in the field are still few. This is mainly because the suppressiveness abilities of composts are difficult to predict from one year to another despite using similar raw materials and composting facilities (Raviv 2008).

As a part of an Indo-Finnish joint project, twenty one commercially produced composts in Finland were screened for their ability to suppress plant disease caused by *Phytophthora cactorum* (Lebert & Cohn) J. Schröt. and *Pythium* spp. About one third of the composts demonstrated suppressiveness against either disease in a plant bioassay (Vestberg et al. 2011). The aim of this work was to study the reproducibility of suppressiveness against *Pythium* wilt on cucumber (*Cucumis sativus* L.) when using composts produced by the same composting plant in two successive years.

## Material and methods

### Composts

The study included nine commercial composts produced in 2008 and 2009. The choice of the nine composts was based on the results of two larger screening experiments including 21 composts that were carried out against *Pythium* wilt in cucumber and *Phytophthora cactorum* crown rot in strawberry (*Fragaria x ananassa* Duchesne) (Vestberg et al. 2011). Five composts were suppressive, three neutral while one suppressed *Pythium* wilt in the previous study. Six composts were produced in closed systems like tunnels or drums followed by maturation in windrows while three composts were produced in windrows from the outset (Table 1). The raw materials varied considerably. There was one poultry manure (PM) compost, four biowaste composts (BW1, BW2, BW3 and BW4), one sewage sludge (SS) compost and one compost of each of the following mixtures: horse manure + paper mill sludge (HM + PMS), cattle manure + garden waste (CM+GW) and sewage sludge + biowaste (SS+BW) (Table 1). The first compost samples from 6–9 month old windrows were collected in April–May 2008, and were thereafter stored in the dark at +4 °C until use in the experiment established in August, 2009. During the storage time nitrification occurred in all composts (results not shown). The second sampling was carried out in the same production plants in April–May 2009, and thereafter stored at +4 °C. From the compost windrows, five compost samples were dug from 10–50 cm depth and thereafter pooled to give one composite sample. Details about the composting process itself such as duration, temperature curves and number of turnings at the various production sites were not available.

### Chemical analyses

Chemical properties of composts were given by the compost producers, including pH, electrical conductivity (EC), total amounts of nitrogen ( $N_{\text{tot}}$ ) carbon (C) phosphorus ( $P_{\text{tot}}$ ) and potassium (K). In addition, water-soluble concentrations of nitrate ( $\text{NO}_3$ ) and ammonium ( $\text{NH}_4$ ) were measured (EN 13652). Ammonium-acetate-extractable amounts of P and K in composts were measured according to Finnish standard tests (Vuorinen and Mäkitie 1955). At harvest of the experiment, samples from the various treatments were analysed for their pH, EC and amounts of available P, K, magnesium (Mg) and Calcium (Ca).

Compost maturity was estimated in two ways. Composts with a  $\text{NO}_3\text{-N}/\text{NH}_4\text{-N}$  ratio exceeding the value 1 were regarded as mature (Itävaara et al. 2006). Compost maturity was also measured by the Rottegrad test, which is a test of self-heating for composts developed by the The German Federal Compost Quality Assurance Organization. The test is performed in an open Dewar vessel (1.5l). Temperature is measured in the lower third of the vessel, for at least 5 days, and the maximum temperature is recorded. The rotting degrees are assigned I ( $T_{\text{max}}$  60–70°C) to V ( $T_{\text{max}}$  20–30°C). Compost with rotting degrees II or III is designated as fresh compost, and a rotting degree of IV or V indicates mature compost.

Table 1. Types, raw materials used and maturity of composts produced in 2008 and 2009.

| Compost                                   | Type of composting | Ratio<br>NO <sub>3</sub> -N/NH <sub>4</sub> -N |      | Rottegrad |      |
|---|--------------------|--|------|-----------|------|
|   |                    | 2008   | 2009 | 2008      | 2009 |
| PM = Poultry manure                       | Windrow            | -  | 0.3  | V         | V    |
| BW1 = Biowaste 1                          | Windrow            | 0.5  | 2.1  | V         | III  |
| HM+PMS = Horse manure + paper mill sludge | Drum               | 1.0  | 0.0  | V         | II   |
| CM+GV = Cattle manure + garden waste      | Windrow            | >4.3   | 280  | V         | V    |
| BW2 = Biowaste 2                          | Drum + tunnel      | >1.2   | 120  | V         | V    |
| BW3 = Biowaste 3                          | Tunnel             | 0.5  | 182  | V         | V    |
| SS=Sewage sludge                          | Tunnel             | >1.8   | 3.2  | V         | V    |
| SS+BW = Sewage sludge + biowaste          | Tunnel             | >2.8   | 463  | V         | V    |
| BW4 = Biowaste 4                          | Tunnel             | 6.5  | 9.1  | V         | V    |

### Experimental set-up

The experiment was a strip-strip-plot design with year as main strip, *Pythium* inoculation as sub-strip and compost as strip-strip-plot. It was arranged on greenhouse tables in 5 blocks. Cucumber seedlings were grown for ten days prior to establishment of the experiment. Two *Pythium* strains, a Finnish *Pythium* sp. originating from *Zantedeschia* sp. and a *P. ultimum* (CBS 101588, origin tomato) strain, were used together as inoculants. The fungi were cultivated on potato dextrose agar (PDA) plates for one week at room temperature prior to inoculation. To inoculate the plants, the agar medium, including the fungal mycelium, was ground and mixed. The inoculum was added to the pots at planting depth (about 5 cm), 5 g of inoculum strain<sup>-1</sup>. Ground PDA medium alone was added to the control pots as 10 g pot<sup>-1</sup>. The plants were grown between 27 August and 30 September 2009 in a greenhouse at MTT Agrifood Research Finland, Jokioinen, Finland, at temperatures of 24°C (day) and 18°C (night) with a 16 hour day length. Prior to the establishment of the experiment, both *Pythium* strains were tested and found capable of causing growth decrease and wilting symptoms in cucumber.

The composts were used as 20% mixtures (v:v) with a steam sterilized (on three successive days) light natural *Sphagnum* peat and sand (3:1, v:v). Depending on the pH in the compost, the basic peat substrate was limed at a rate of 0–7 g Dolomite lime l<sup>-1</sup> peat to reach a pH of 6–6.5. The amounts of soluble nutrients in the compost mixes were targeted to about 250 mg N, 100 mg P and 350 mg K l<sup>-1</sup> compost mix by adding compound fertilizers of various nutrient composition. Two controls without compost were established. These had the same basic substrate as the compost containing mixtures; one of the controls was steam sterilized while the other one was not. The compost was replaced by dark peat in the controls. Based on the bulk densities of composts (0.5–0.7 g cm<sup>-3</sup>), light (0.06 g cm<sup>-3</sup>) and dark (0.11 g cm<sup>-3</sup>) *Sphagnum* peat and sand (1.4 g cm<sup>-3</sup>), the bulk densities of the final mixtures were roughly estimated to 0.42–0.47 g cm<sup>-3</sup> and 0.34 g cm<sup>-3</sup>, for mixtures containing composts or being without composts, respectively. During the experiment, the plants were fertigated weekly with a 2 mS cm<sup>-1</sup> compound fertilizer solution (14,7N–5P–21K, Yara Ltd.).

### Plant growth and disease assessment

Plant height was measured weekly starting one week after planting. At harvest, plant dry weight (DW) was also measured. Suppressive capacity (SC) was calculated separately for each compost as the difference (%) in DW between *Pythium* inoculated and non-inoculated plants.

At harvest disease severity was assessed as median differences in plant vigour, (on a scale 0–5, 0 = dead, 5 = very good), leaf colour (on a scale 1–3, 1 = highly discoloured, 3 = dark green) and discoloration of the root system (on a scale of 1–3, 1 = poor, 3 = good).

### Statistical analyses

The analysis of variance was based on the common mixed model for a strip-strip plot. Fixed factors were compost origin, sampling year and disease inoculation, whereas replication was as a random (blocking) factor. In the case of SC values, the model was reduced to a strip plot since the difference between inoculated and un-inoculated treatments was calculated and the effect of disease inoculation could not be tested separately. A suitable covariance structure was chosen by comparing compound symmetry and heterogeneous compound symmetry structure against unstructured covariance using a likelihood-ratio test.

Pairwise comparisons were performed using two-sided t-type tests. Model assumptions were checked graphically. Equality of variances was visually judged by plotting residuals against fitted values and normality of the variables by inspecting model residuals in a normal probability plot. Statistical analyses were performed using the SAS system, Enterprise Guide version 4.2 (SAS Institute Inc. 2008).

The examination of the model residuals revealed two influential outliers for the SC variable. *Pythium* inoculation increased shoot DW by 101% and 51% in these two cases, respectively. The influence of the outlying values on the results was examined by comparing results of the analysis of the reduced and complete data. On checking the data, no logical reason for the exceptional values was determined. It was possibly an issue of human error. We decided to use the reduced data in the statistical analysis of SC.

## Results

### Compost properties

Most of the composts were mature according to the  $\text{NO}_3/\text{NH}_4$  ration and the Rottegrad test (Table 1). The  $\text{NO}_3/\text{NH}_4$  ration however indicated immaturity of two composts produced in 2008 and 2009. According to the Rottegrad test, no composts produced in 2008 were immature while two composts produced in 2009 were so.

The total amounts of nutrients in composts from 2008 and 2009 did not differ much from each other (Table 2). The carbon content was, however, considerably lower in one compost (HM+PMS) produced in 2009 compared with the compost produced in 2008. Compared with 2008 samples, the total amounts of P were higher in 2009 samples in two composts (HM+PMS and SS composts) and those of K in three composts (HM+PMS, BW1 and BW3 composts). The pH of composts varied considerably from about 5 to more than 8. The pH was several units higher in the HM+PMS compost in 2009 than in 2008 (Table 3). The EC was clearly higher in 2009 in four out of nine composts. On the other hand, the PM compost had lower EC in 2009 than in 2008 (Table 3). The amounts of available N, P and K varied more between production year than did the levels of total nutrients (Table 2 and 3).

Table 2. The amounts of total nitrogen, carbon, phosphorus and potassium in nine compost lots produced in 2008 and 2009 at commercial compost producing plants in Finland. PM=poultry manure, BW=biowaste, HM=horse manure, PMS=paper mill sludge, CM=cattle manure, GW=garden waste, SS=sewage sludge, DW=dry weight. A number after BW indicates different producers.

| Type of compost | Total nutrients, % |      |        |      | Total nutrients, g kg <sup>-1</sup> compost DW |      |           |      |
|-----------------|--------------------|------|--------|------|--|------|-----------|------|
|                 | Nitrogen           |      | Carbon |      | Phosphorus                                     |      | Potassium |      |
|                 | 2008               | 2009 | 2008   | 2009 | 2008   | 2009 | 2008      | 2009 |
| PM              | 3.2                | 3.5  | 41.1   | 41.2 | 17.9   | 17.1 | 33.3      | 32.6 |
| BW1             | 3.7                | 3.5  | 44.8   | 43.4 | 3  | 4.0  | 2.7       | 7.3  |
| HM+PMS          | 1.7                | 1.8  | 47.5   | 33.1 | 3.2  | 8.7  | 3.6       | 17.4 |
| CM+GW           | 1.4                | 1.3  | 24.0   | 23.3 | 2.7  | 3.6  | 4.8       | 6.3  |
| BW2             | 1.8                | 1.6  | 32.8   | 28.0 | 3.2  | 3.5  | 9.5       | 8.5  |
| BW3             | 2.8                | 3.2  | 33.7   | 29.5 | 7.1  | 8.1  | 6.9       | 15.5 |
| SS              | 1.9                | 2.3  | 28.1   | 25.9 | 21.6   | 25.2 | 1.8       | 1.9  |
| SS+BW           | 2.7                | 2.8  | 29.3   | 27.3 | 29.2   | 30.8 | 5.7       | 5.2  |
| BW4             | 2.1                | 2.5  | 23.5   | 25.6 | 4.7  | 6    | 13.6      | 14.4 |

The attempt to adjust pH and levels of available nutrients in compost mixtures close to each other by using compound fertilizers was partly successful. At the end of the cucumber bioassay, the pH levels of substrate mixtures of composts collected in 2008 varied within 1 unit and those of composts collected in 2009 within 1.6 units (Table 4). Corresponding values for the original composts were 3.1 and 3.2 units, respectively (Table 3). The amount of available Ca, K, Mg and P also varied considerably. The greatest variation was found in P, where values ranged between 27 and 487 and between 30 and 360 in mixtures of composts produced in 2008 and 2009, respectively.

Table 3. pH, electrical conductivity and the amounts of available ammonium, nitrate, phosphorus and potassium in nine compost lots produced in 2008 and 2009 at commercial compost producing plants in Finland. PM=poultry manure, BW=biowaste, HM=horse manure, PMS=paper mill sludge, CM=cattle manure, GW=garden waste, SS=sewage sludge, EC=electrical conductivity, N=nitrogen. A number after BW indicates different producers.

| Compost<br>Nr | pH   |      | EC<br>mS cm <sup>-2</sup> |      | Available nutrients, mg l <sup>-1</sup> compost |      |           |      |            |       |           |      |
|---------------|------|------|---------------------------|------|---|------|-----------|------|------------|-------|-----------|------|
|               | 2008 | 2009 | 2008                      | 2009 | Ammonium-N                                      |      | Nitrate-N |      | Phosphorus |       | Potassium |      |
|               |      |      |                           |      | 2008  | 2009 | 2008      | 2009 | 2008       | 2009  | 2008      | 2009 |
| PM            | 7.8  | 8.3  | 3.7                       | 1.2  | -   | 378  | -         | 124  | 1048       | 1325  | 4837      | 5250 |
| BW1           | 6.0  | 5.5  | 0.2                       | 1.5  | 81  | 89   | 37        | 183  | 90.5       | 184.4 | 218       | 1284 |
| HM+PMS        | 5.3  | 8.4  | 0.5                       | 2.5  | 81  | 643  | 85        | 0    | 28.7       | 239.5 | 346       | 3010 |
| CM+GW         | 7    | 8.1  | 0.8                       | 0.5  | <78   | 0    | 339       | 28   | 68         | 58.7  | 603       | 484  |
| BW2           | 8.2  | 8.0  | 1.4                       | 0.9  | <78   | 2    | 96        | 239  | 3.9        | 2.04  | 1318      | 760  |
| BW3           | 7.5  | 7.5  | 0.5                       | 2.3  | 159   | 2    | 73        | 364  | 154.7      | 57.1  | 652       | 3266 |
| SS            | 5.1  | 5.4  | 1.3                       | 2.3  | <78   | 350  | 142       | 1111 | 1.9        | 1.39  | 102       | 137  |
| SS+BW         | 5.4  | 5.2  | 1.2                       | 0.9  | <78   | 1    | 222       | 463  | 3.4        | 4.38  | 520       | 313  |
| BW4           | 7.8  | 8.2  | 4.0                       | 2.9  | 81  | 39   | 524       | 356  | 5.9        | 8.33  | 3215      | 2759 |

Table 4. pH, and the amounts of available calcium (Ca), potassium (K), magnesium (Mg) and phosphorus (P) in nine compost mixtures and in sterilized and natural peat controls at harvest of a cucumber bioassay in the greenhouse with Finnish commercial composts produced in 2008 and 2009. PM=poultry manure, BW=biowaste, HM=horse manure, PMS=paper mill sludge, CM=cattle manure, GW=garden waste, SS=sewage sludge, EC=electrical conductivity, N=nitrogen. A number after BW indicates different producers.

| Treatment          | pH   |      | Ca, mg l <sup>-1</sup><br>substrate |      | K, mg l <sup>-1</sup><br>substrate |      | Mg, mg l <sup>-1</sup><br>substrate |      | P, mg l <sup>-1</sup><br>substrate |       |
|--------------------|------|------|-------------------------------------|------|------------------------------------|------|-------------------------------------|------|------------------------------------|-------|
|                    | 2008 | 2009 | 2008                                | 2009 | 2008                               | 2009 | 2008                                | 2009 | 2008                               | 2009  |
| PM                 | 5.73 | 5.74 | 1872                                | 1685 | 538                                | 620  | 608                                 | 655  | 487.0                              | 359.7 |
| BW1                | 6.03 | 6.3  | 1981                                | 1891 | 347                                | 304  | 769                                 | 722  | 42.6                               | 44.0  |
| HM+PMS             | 6.09 | 5.94 | 1112                                | 2364 | 291                                | 391  | 497                                 | 287  | 29.4                               | 151.3 |
| CM+GW              | 5.96 | 5.44 | 1579                                | 1253 | 243                                | 274  | 517                                 | 381  | 41.4                               | 61.9  |
| BW2                | 6.28 | 6.94 | 2548                                | 3472 | 489                                | 577  | 349                                 | 396  | 47.2                               | 69.2  |
| BW3                | 6.25 | 6.22 | 2568                                | 2603 | 362                                | 412  | 581                                 | 556  | 206.4                              | 199.6 |
| SS                 | 5.94 | 5.64 | 1582                                | 1843 | 246                                | 203  | 476                                 | 435  | 26.8                               | 29.7  |
| SS+BW              | 5.68 | 5.72 | 1393                                | 1510 | 299                                | 252  | 478                                 | 565  | 28.6                               | 26.7  |
| BW4                | 6.7  | 6.97 | 4726                                | 4963 | 648                                | 742  | 382                                 | 399  | 185.3                              | 230.6 |
| Sterilized control |      | 6.03 |                                     | 1646 |                                    | 409  |                                     | 562  |                                    | 48.1  |
| Natural control    |      | 5.65 |                                     | 1942 |                                    | 309  |                                     | 525  |                                    | 39.8  |

### Suppressiveness against *Pythium* wilt

By the end of the experiment, *Pythium* inoculation had decreased cucumber plant height by 10.4% in control substrate mixtures without addition of compost (Figure not shown). Weekly measurement of plant height indicated different curves for suppressive composts than for non-suppressive ones (Fig 1). In the former case (for example in composts BW1, HM+PMS and CM+GW), *Pythium* inoculated and non-inoculated curves remained very close to each other, indicating that the pathogen did not cause growth decrease when assessed as plant height. Also in BW2, suppressiveness seemed to occur similarly in both samples despite different growth of cucumber during the two years. Compost PM, on the other hand, encouraged similar growth in both years, but the effect of compost was conducive rather than suppressive.

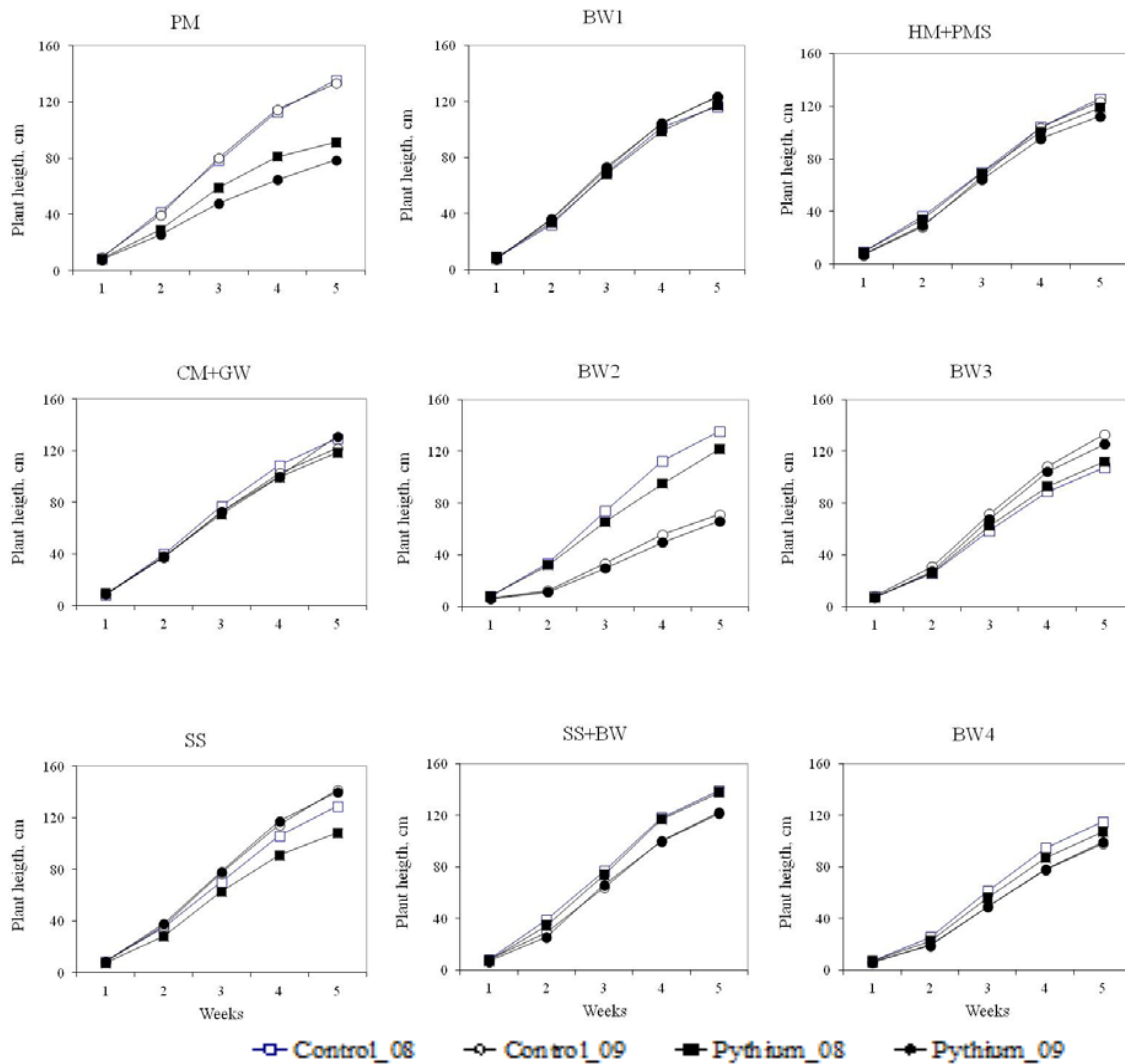


Fig. 1. Impact of inoculation with *Pythium* vs control on plant height, measured weekly, of cucumber in growing media containing 20% compost produced in 2008 or 2009. PM=poultry manure, BW=biowaste, HM=horse manure, PMS=paper mill sludge, CM=cattle manure, GW=garden waste, SS=sewage sludge, DW=dry weight. A number after BW indicates different producers.

Analysis of variance showed that *Pythium* inoculation significantly decreased cucumber DW. For this parameter, the main effects of Compost and Year, as well as the interactions Compost x *Pythium*, Compost x Year and Year x *Pythium* were statistically significant (Table 5). Because the varying nutrient levels in different compost mixtures caused differences in growth, the parameter “difference in shoot DW between *Pythium* inoculated and non-inoculated” (the suppressiveness capacity, SC), is better suited for the comparison of composts. The Year of production did not significantly affect this variable.

Table 5. Analysis of variance results for the *Pythium* wilt experiment. Measured variables were dry weight of cucumber plants at the end of the experiment and the percentage difference in shoot DW between non-inoculated and *Pythium* inoculated.

| Effect                          | Shoot dry weight (DW) |        |         |         | Shoot DW difference, non-inoculated – <i>Pythium</i> inoculated |        |         |         |
|---------------------------------|-----------------------|--------|---------|---------|---|--------|---------|---------|
|                                 | Num DF                | Den DF | F value | Pr>F    | Num DF  | Den DF | F value | Pr>F    |
| <i>Pythium</i>                  | 1                     | 4      | 23.03   | 0.0087  |   |        |         |         |
| Compost                         | 10                    | 77.9   | 9.27    | <0.0001 | 10  | 72     | 5.73    | <0.0001 |
| Year                            | 1                     | 50.2   | 27.27   | <0.0001 | 1   | 4.5    | 0.1434  | 0.1434  |
| <i>Pythium</i> x compost        | 10                    | 72.4   | 4.45    | <0.0001 |   |        |         |         |
| <i>Pythium</i> x year           | 1                     | 41.9   | 4.18    | 0.0473  |   |        |         |         |
| Compost x year                  | 10                    | 50.2   | 24.02   | <0.0001 | 8   | 72     | 0.5023  | 0.5023  |
| <i>Pythium</i> x compost x year | 10                    | 41.9   | 1.57    | 0.1491  |   |        |         |         |

The SC of individual composts is shown in Figure 2. In steam-sterilised peat without compost, shoot DW decreased by 32.7% following *Pythium* inoculation. Growth decrease in the majority of composts was significantly lower than this. An exception was the poultry manure (PM) compost, which caused a slight increase in *Pythium* wilt. Similarly to the majority of composts, the natural peat substrate also significantly decreased *Pythium* disease.

The impact of *Pythium* inoculation on the visually estimated cucumber variables of plant vigour, leaf colour and root discoloration measured at harvest was negligible, so those results are not presented here.

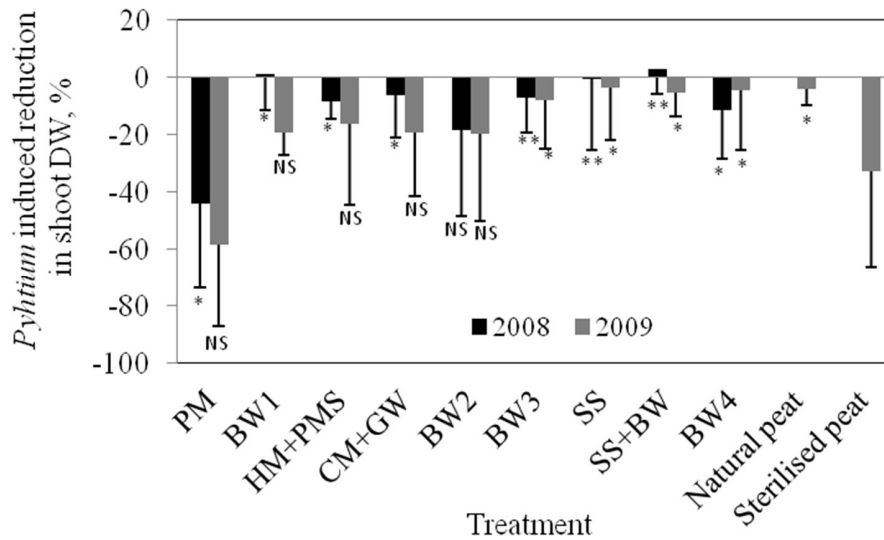


Fig. 2. Impact of composts produced in two successive years (2008 and 2009) on *Pythium* induced reduction (*Pythium* inoculated – non-inoculated, %) in shoot dry weight of cucumber measured at harvest of a pot experiment conducted in greenhouse conditions of 5 weeks duration. Lines on bars indicate standard deviation of means (N=5). PW=poultry manure, BW1 – BW4 = biowaste 1 – biowaste 4, respectively, HM=horse manure, PMS=paper mill sludge, SS=sewage sludge. A number after BW indicates different composting plants using this raw material. Bars indicated with \* and \*\* differ from the sterilized peat control at  $p < 0.05$  and  $0.01$ , respectively. Bars indicated with NS do not differ from the sterilized peat control.

## Discussion

Suppression of soil-borne disease by using composts has been reported in numerous studies (recently reviewed by Noble 2011), but the practical applications of the phenomenon remain limited. The main reason for this is the lack of reliable prediction and quality control tools for evaluation of the level and specificity of the suppression effect (Hadar and Papadopoulou 2012). We must also bear in mind that composts are living substrates that will probably never completely reach the stability found in manufactured products like inorganic fertilizers. The variations in compost properties are due to the choice of raw materials, their proportions in the compost mix, and the temperature regime and moisture control during composting (Raviv 2013). The difficulties in producing compost of stable quality during two or more successive years is maybe the biggest bottleneck preventing the large scale use of composts in high-input agriculture and horticulture.

We compared the chemical and biological quality of nine composts produced during two successive years (2008 and 2009). Compost raw materials and composting systems were the same during both years. Despite this, nutritional and maturity differences did occur between composts produced in 2008 and 2009. Compost maturity has been shown to be a prerequisite for the development of suppressiveness (Hoitink & Fahy 1986, Kuter et al. 1988, Hadar and Gorecki 1991) in most studies. The impact of compost maturity was verified also in our study. The two immature composts from 2009 were not suppressive while the corresponding mature composts from 2008 were (Table 1, Fig. 2). These results are in line with the findings of Chef et al. (1983), who showed that green composted hardwood bark was conducive to *Fusarium* wilt while the mature composted hardwood bark was suppressive.

Although some nutritional and maturity differences were evident between composts produced in 2008 and 2009, the overall SC (suppressive, neutral or conducive) expressed as the percentage difference in cucumber shoot DW between non-inoculated and *Pythium* inoculated plants was not affected by year of production (Table 5, Fig 2). This result is encouraging, indicating that the suppressiveness can be reproduced in commercial composting plants if the raw materials and the composting method are exactly the same. In other studies, reproducibility has been achieved in experiments when the same composts were used in different experiments (Serra-Wittling et al. 1996, Widmer et al. 1998, Borrero et al. 2004). Reports on comparisons of composts produced during two or more successive years seem not to be available.

Six month longer storage of the 2008 composts at 4 °C did not lower their SC, as judged from the fact that the SC was largely similar to that for the previous (Vestberg et al. 2011) cucumber wilt experiment. In other studies, storage of composts has not affected suppressiveness or has led to loss of suppressiveness. Saadi et al. (2010) demonstrated that compost suppressiveness against *Fusarium* wilt of melon can be maintained for at least one year under a wide range of storage conditions, without any loss of suppressive capacity. Van Rijn et al. (2007) found that the effect of storage on suppression of *Fusarium* wilt on flax (*Linum usitatissimum* L.) was compost dependent. In most cases, three months of storage did not affect suppression, but for one compost all three storage methods studied (at +4 °C, at –20 °C or as dry) eliminated the 24% disease suppression recorded for the fresh compost. In some other composts, storage has even caused a significant increase in disease suppression (van Rijn et al. 2007). In a study of Widmer et al. (1998), addition of fresh municipal waste compost reduced *Phytophthora nicotianae* infection in citrus seedlings, but this effect was lost after storage. Suppressiveness of a leaf compost against *Pythium* damping-off of cotton was not affected by 10 years of storage in a study of McKellar and Nelson (2003).

As compared with a steam sterilized control, also the natural light *Sphagnum* peat control significantly decreased *Pythium* disease in our study. This result is in agreement with the results of Tahvonon (1982) and Wolffhechel (1988), who found that some lots of light *Sphagnum* peat had suppressed *Pythium* spp. This phenomenon was further verified by Boehm and Hoitink (1992), who found suppression of *P. ultimum* when using light coloured peat of the quality H2 on the von Post decomposition scale. The impact of peat changed to conduciveness when more decomposed peat was used (H4 on the von Post scale). In contrast to this result is the finding of Hunter et al. (2006), who established no correlation between the level of peat decomposition (H2–H5) and disease suppression of *P. sylvaticum* in cress. Wolffhechel (1988) concluded that there is a microbiological reason behind the suppressiveness of peat because the effect could be destroyed by heat treatment and by addition of benomyl. According to Tahvonon (1982), strongly pathogen antagonistic strains of *Streptomyces* and *Trichoderma viride* can be isolated from suppressive peat.

The choice of parameters for showing suppressiveness is important, in particular when working with pathogen inoculation in a bioassay. In our case, few cucumbers wilted and died as a result of *Pythium* inoculation, so it was not possible to compare the composts by creating a disease index. Leaf colour and general shoot vigour were not good indicators of the disease either. However, *Pythium* caused a clear reduction of shoot growth that was best recognised in the dry matter accumulation (33% lower) and to some extent also in plant height (10% lower). Researchers often meet problems when trying to adjust suitable levels of disease outbreak in bioassays. The result from pathogen inoculation is easily sudden death of all test plants. From this point of view, we consider the clear decrease in dry matter of cucumber as a good indicator of the functioning of the pathogen.

We used compost at the rate of 20% v/v of the growing medium. According to Raviv (2008), compost amendment at the rate of 10–25% of the growing medium has been enough to induce suppressiveness in the majority of studies. Tuitert et al. (1998) pointed out that the dosage of composts applied in potting mixes with peat is limited to 20% at maximum also due to their high salt content. In our case, it would not have been possible to increase the rates of composts because of very high levels of available P in some composts. However, Veeken et al. (2005) showed that the disease suppressiveness of potting mixes strongly increased from 31 to 94% when the compost amendment rate was increased from 20 to 60%. They used biowaste compost that was wet sieved prior to composting. In this way, they achieved a high quality compost that was high in organic matter, but low in EC and heavy metals. Such quality composts may well be used in potting mixes at concentrations considerably higher than 20% (Veeken et al. 2005).

It can be concluded that the reproducibility of the SC was good in this scientific study, but in practice variations in compost quality often lead to variations in SC. Because of this growers are still reluctant to rely on compost to control soil-borne disease (Pugliese et al. 2011). Two approaches can be taken to increase the level of SC of composts. First, reproducible composting techniques that produce composts with predictable chemical, physical and



biological quality and high suppressive capacity, should be adopted. Second, the suppressive capacity can be ensured and further increased by inoculation of the composts with biological control agents (BCAs). The latter possibility has been investigated in a number of studies, of which there has been a significant increase in the suppressive effect of compost by the addition of BCAs (Noble et al. 2006). In the majority of studies, *Trichoderma* BCAs have been used for increasing the suppressive effect (Hoitink 1990, Trillas et al. 2006, Pugliese et al. 2011, Bernal-Vicente et al. 2012), but positive effects from non-pathogenic *Fusarium* (Postma et al. 2003) and *Bacillus* (Nakasaka et al. 1998) have also been observed. Scheuerell et al. (2005) concluded that currently available composts could potentially provide commercially acceptable control of *Pythium* spp., but it is necessary to fortify composts with BCAs for the control of *Rhizoctonia solani*.

## Conclusions

Production year of nine commercial Finnish composts did not affect their suppressive capacity (whether suppressive, intermediate or conducive) to *Pythium* wilt of cucumber studied in a bioassay. Natural light *Sphagnum* peat also suppressed the disease. Slight differences in nutrient levels and pH between composts from 2008 and 2009 did not affect the SC, but the SC was decreased in two immature composts of 2009. Six months of storage of the 2008 composts at +4 °C did not affect the SC of the composts compared with results from a previous experiment.

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