# Dry Mycelium of *Penicillium chrysogenum* Induces Resistance Against Verticillium Wilt and Enhances Growth of Cotton Plants

Hezhong Dong[1,2] and Yigal Cohen[1,*]

Dry mycelium (DM) of *Penicillium chrysogenum* and its water extract (DME) were examined for their effects on induced resistance against Verticillium wilt and plant growth of cotton in the greenhouse. Soil application of 0.1–5% DM or 0.5–5% DME provided significant protection against the wilt, relative to the control. As neither DM nor DME inhibited mycelial growth of *Verticillium dahliae in vitro*, it is suggested that the disease-controlling effects of DM or DME are attributed to induced resistance. DME (5%), as well as DME treated with chloroform or cold acetone, were as effective as 2% DM in reducing disease severity of Verticillium wilt, implying that the resistance-inducing substance(s) in DM are mostly water-soluble, with neither proteins nor lipids likely to be responsible for the induction of resistance. No significant difference in root colonization with *V. dahliae* was found between control-inoculated and 2% DM- or 5% DME-inoculated plants. However, colonization of hypocotyls and epicotyls was drastically suppressed by either 2% DM or 5% DME relative to the control. Treatments with 2% DM or 5% DME significantly increased ionically-bound peroxidase (POX) activity in roots, hypocotyls and the second leaf of cotton plants, with the hypocotyls expressing the highest increase. Soil application of DM or DME increased plant height, fresh and dry weight of inoculated and non-inoculated cotton plants, relative to their corresponding controls. It is concluded that DM may be used in cotton crops to promote plant growth and to induce resistance against *V. dahliae*. POX might be associated with the defense against Verticillium wilt.

KEY WORDS: *Verticillium dahliae*; peroxidase activity; *Gossypium hirsutum; Gossypium barbadense.*

## INTRODUCTION

Verticillium wilt of cotton is an economically important disease that occurs in most cotton production areas of the world (12,36). *Verticillium dahliae* Kleb., the causal organism of this disease, is a soilborne fungus which penetrates the cotton root and systemically infects the plant through the xylem (9,15). Infected cotton plants usually exhibit symptoms of marginal chlorosis or necrosis in leaves, discoloration of the stem vascular bundles, decrease in photosynthesis and increase in respiration, thus resulting in a significant reduction of the plant biomass and heavy loss of yield (14). The disease is

Received Aug. 1, 2001; received in final form Nov. 24, 2001; http://www.phytoparasitica.org posting Jan. 9, 2002.

[1]Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel. *Author for correspondence [Fax: +972-3-5354133; e-mail: coheny@mail.biu.ac.il].

[2]On sabbatical leave from Cotton Research Center, Shandong Academy of Agricultural Sciences, Jinan 250100, China.

generally managed by using wilt-resistant cultivars, crop rotation and cultural practices (2,27,36). However, none of the genetic sources of resistance actually provides protection from infection of the vascular system, and none of the current upland cotton cultivars is immune to *V. dahliae*, although some are more tolerant than others (6). Since the pathogen *V. dahliae* is able to survive in the residue of many host plants and remains viable in soil for years, it is also difficult to control it by cultural practices alone (28). Therefore, it is necessary to develop additional methods.

Enhancement of the natural defense systems of plants in order to provide resistance against fungal, viral, bacterial and nematodal pathogens is a newly developed approach for plant protection (5,22,25). Local and systemic resistance can be induced by biological or chemical means in a number of plant species (23,26) including cotton. It has been reported that 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) are able to induce resistance against Alternaria leaf spot of cotton (4,7). Presoaking of seed in BTH and application of the biological control agent *Trichoderma virens* induced systemic resistance against, respectively, *Fusarium oxysporum* f.sp. *vasinfectum* and *Rhizoctonia solani* in cotton during the early stage of growth (1,6,17).

The phenomenon of induced resistance in cotton against Verticillium wilt was first demonstrated by prior inoculation with a mild or an avirulent strain of the same pathogen (3,29). Later, resistance against *V. dahliae* was induced with the aid of spider mites (21), elicitors from *V. dahliae* (11), and chemical compounds such as DL-3-aminobutyric acid (3-ABA), methyl jasmonate (MeJA), INA and BTH (6,24), as well as live *T. virens* (15). Here, we report induced resistance against Verticillium wilt in cotton by dry mycelium (DM), a dry fungal biomass of *P. chrysogenum* obtained from industry after extraction of penicillin. *Penicillium chrysogenum* was reported to be a potent biological control agent against *Botrytis fabae* (18). When amended to the soil, DM enhanced plant growth by adding nutrients and improving soil fertility (16), and protected corn plants by decreasing the density of *Fusarium moniliforme* in soil (13). Recently, we found that an aqueous extract of DM induced resistance against *Fusarium oxysporum* f.sp. *melonis* in melon (10).

The objectives of this research were to examine the effects of DM and its water extract on induction of resistance against Verticillium wilt and plant growth of cotton. The activities of cytoplasmic and ionically-bound peroxidases involved in the induction of resistance were also studied.

## MATERIALS AND METHODS

**Preparation of dry mycelium extracts** Dry mycelium (powder) of *P. chrysogenum* was obtained from Biochemie Ltd., Kundl, Austria. This fungal biomass, dried by the manufacturer for 4 h at 110°C, contains no penicillin. It is marketed as an organic fertilizer (N: $K_2O$: $P_2O_5$= 7:1:2). Water extract of dry mycelium (DME) was prepared using the following procedure: 100 g of DM (powder) was suspended in 1000 ml distilled water. The suspension was shaken for 2 h at 100 rpm and then stored for 22 h at room temperature. Afterwards it was briefly agitated and filtered through Whatman No. 1 paper. The filtrate was autoclaved for 30 min at 110°C and the pH measured after cooling (10% DME, pH =2.6–2.8). DME (10%) was stored as stock solution at 4°C. Chemical analysis of 5% DME revealed 570 ppm total N, 186 ppm $P_2O_5$ and 650 ppm $K_2O$.

Lipid-free DME and protein-free DME were prepared with the aid of chloroform and cold acetone, respectively. One hundred ml DME (10%) was mixed and thoroughly

agitated with 100 ml chloroform for 5 min. When two phases formed after 24 h, the upper water phase was collected, its chloroform residues were dried off at 40°C, and a lipid-free DME was thus obtained as the residue. The residue was then dissolved in water with the aid of a droplet of Tween 20. One hundred ml DME (10%) was mixed with 100 ml cold (-20°C) acetone. After 24 h on ice, the mixture was centrifuged at 10,000 *g* for 30 min at 4°C. The protein sediment was discarded. Protein-free DME was obtained from the supernatant after evaporation of the residue acetone at 40°C.

**Plant culture and DM or DME treatment** Two cotton cultivars, H552 (*Gossypium hirsutum* L.) and PF15 (*Gossypium barbadense* L.), obtained from Hazera Genetics, Israel, were used in this study. Seeds were sown in 1000-ml pots containing an autoclaved mixture of perlite and peat (1:1, v/v). After emergence, four healthy seedlings were left in each pot and allowed to grow in the greenhouse (20–32°C). When plants reached the two-leaf stage (13–15 days after emergence), 0.5–5% DME or 0.5% fertilizer (20:20:20, N:P:K) was drenched into each pot (50 ml per pot). Plants drenched with an equal volume of distilled water were used as controls, unless indicated otherwise.

For the DM (powder) treatment, DM (0.1–5%, w/w) or fertilizer (0.5%, w/w) was amended to the potting mixture of perlite and peat, autoclaved, potted and then watered. Seeds were sown in each pot one week afterwards. Seedlings were allowed to grow in the greenhouse under the conditions described above. After emergence, plants were watered every 2 or 3 days with an equal volume of water per pot.

**Fungal inoculum and inoculation** A highly aggressive strain of *V. dahliae* (DL2) (obtained from Dr. Talma Katan, The Volcani Center, Agricultural Research Organization, Bet Dagan, Israel) was grown in 9-cm-diam petri dishes on 50% PDA at 25°C in the dark. Twenty-day-old cultures were crushed in sterile water and the conidial concentration was adjusted to $\sim 10^6$ per ml before inoculation, unless indicated otherwise. DME-treated plants were inoculated 3 days after treatment and DM-treated plants at 15–18 days after emergence. For inoculation, plants were carefully removed from soil and thoroughly washed with water. The root tips (3 cm) were cut off, the root system was dipped in the inoculum for 5 min, and inoculated plants were transplanted into their original pots. Pots were placed in a water-saturated atmosphere inside transparent plastic boxes for 2 days to avoid loss of turgor. Plants were then allowed to grow in the greenhouse under controlled temperatures (20/28°C, night/day) and a 12-h photoperiod.

**Assessment of disease severity and plant growth** Disease severity of Verticillium wilt was evaluated 3–4 weeks after inoculation using a diseased plant index (DPI), leaf wilt index (LWI) and vascular discoloration index (VDI), according to Li *et al.* (24). DPI = number of diseased plants/ total number of inoculated plants; LWI = number of wilting leaves per treatment/total number of leaves in a treatment; and VDI = number of discolored organs (main root, hypocotyls and the first stem node) per treatment/total number of organs in a treatment. Percentage protection was then calculated as (1-*a*/*b*), where *a* is % disease in treated plants and *b* is % disease in control plants. Plant height, fresh weight and dry weight per plant were recorded and statistically analyzed 5 weeks after emergence. All experiments were carried out with five replicates of four plants in each treatment, and repeated three times.

**Colonization of *V. dahliae* in the vascular system** After evaluation of disease severity, one 3-cm section was taken from the main root, hypocotyl and epicotyl of each inoculated

plant (control, treated with 5% DME or 2% DM). Each sampled section was weighed, surface sterilized by dipping in 5% $NaClO_3$ for 30 s, washed with sterile water, and homogenized in 10 ml sterile water. Homogenate was diluted three times with sterile water and dispersed (0.1 ml per plate) on PDA containing 300 $\mu$g $ml^{-1}$ streptomycin sulfate. Plates were incubated at 25°C in the dark for 3 days. Colony forming units (cfu) of *V. dahliae* were counted and the colonization of plants was expressed as cfu $g^{-1}$ fresh weight of plant tissue (24). The experiment was carried out with four replicate plants per treatment and repeated three times.

**Peroxidase activity** Healthy cotton (cv. H552) plants, 3 days after 5% DME treatment or 15 days after emergence in 2% DM, were used for the analysis of peroxidase (POX) activity. Plants were removed from soil, thoroughly washed with water and blotted dry. Root, hypocotyls and the second leaf (0.3–0.6 g) were ground with a mortar and pestle in 10 ml cold 15 mM sodium phosphate buffer (pH 6.0). The suspension was poured into a 50 ml tube, kept still for 30 min in an ice bath, and then centrifuged (10,000 *g* for 20 min at 4°C). The supernatant was used for the analysis of cytoplasmic, soluble POX activity.

Extraction of ionically-bound POX was performed by rehomogenizing the pellet (from each of the above extractions) in 15 mM of sodium phosphate buffer containing 1 M NaCl. Samples were incubated at 4°C for 2 h and centrifuged as described above. The supernatant was used for analyzing the ionically-bound POX activity. POX activity of both soluble and ionically-bound samples was then determined according to the following procedures: Two hundred $\mu$l of the sample extract was added to a mixture of 5 ml 15 mM sodium phosphate buffer (pH 6.0), 100 $\mu$l 0.05 M guaiacol and 100 $\mu$l 2% $H_2O_2$ in a 15 ml tube. The increase in optical density at 470 nm was recorded for 3 min using a Milton Roy Spectronic Genesys 5 spectrophotometer. POX activity was expressed as the change in absorbance per minute per gram fresh weight. Four replicate plants per treatment were used in each experiment. The experiment was repeated twice.

**Fungitoxicity tests** Czapek Dox (CZA) agar containing either 0.5–1% (v/v) DME or 0.5–1% (w/v) DM (powder) was autoclaved, poured into 9-cm-diam petri dishes and inoculated with one mycelium plug (3 mm) of the *V. dahliae* pathogen (eight plates per treatment). The inoculated plates were kept at 25°C in the dark and colony diameter was measured 5 days later. The experiment was carried out twice.

## RESULTS

**Efficacy of DM in controlling Verticilium wilt** Plants treated with 2% or 5% DM, but not those treated with 0.1% DM or mineral fertilizer, were significantly protected – relative to control plants – against the wilt disease. Percentage protection ranged between 40 and 65 (Table 1). The efficacy of DM in disease control was dependent on the concentration applied: at 0.1% it was ineffective and at 5% it provided no better than 2% control. It thus appears that the highest protection against Verticillium wilt was obtained with application of 2% DM (Table 1).

**Efficacy of DME in controlling Verticillium wilt** A soil drench with 0.5–5% DME significantly protected plants of cv. PF15 against the wilt disease, whereas no significant protection was obtained with the application of a mineral fertilizer, relative to water-treated plants (control). Percentage protection by 0.5–5% DM ranged from 24 to 74 (Table 2). Of the concentrations tested, 5% DME provided the greatest protection.

TABLE 1. Efficacy of dry mycelium (DM) of *Penicillium chrysogenum* in controlling Verticillium wilt in cotton cv. H552. Mineral fertilizer (0.5%, w/w) and DM (0.5–5%, w/w) were applied to the soil prior to sowing; plants were inoculated with *Verticillium dahliae* 14 days after emergence; data (means ± SD) were recorded 21 days after inoculation

| Treatment | DPI[z] (%) | % Protection (plants) | LWI[z] (%) | % Protection (leaves) | VDI[z] (%) | % Protection (vascular tissues) |
|---|---|---|---|---|---|---|
| Control | 93.8±12.5 a | – | 45.4±5.3 a | – | 75.0±5.1 a | – |
| Fertilizer | 93.8±12.5 a | 0 | 46.7±6.5 a | 0 | 75.0±9.0 a | 0 |
| 0.1% DM | 95.0±10.0 a | 0 | 43.3±8.2 a | 4.6 | 74.4±8.3 a | 0.8 |
| 2% DM | 43.8±12.5 b | 53.3 | 16.7±5.8 c | 65.2 | 36.0±7.9 b | 52.0 |
| 5% DM | 56.3±12.5 b | 40.0 | 20.0±3.6 c | 56.0 | 37.5±13.5 b | 50.0 |

[z]DPI, diseased plant index; LWI, leaf wilt index; VDI, vascular discoloration index.
[y]Within columns, values followed by the same letter do not differ significantly ($P$=0.05) according to Duncan's multiple range test.

TABLE 2. Efficacy of water extract of dry mycelium (DME) of *Penicillium chrysogenum* in controlling Verticillium wilt in cotton cv. PF15. Mineral fertilizer (0.5%) and DME (0.5–5%) were applied as a soil drench 11 days after emergence; plants were inoculated with *Verticillium dahliae* 3 days after DME treatment; data (means ± SD) were recorded 21 days after inoculation

| Treatment[z] | DPI[z] (%) | % Protection (plants) | LWI[z] (%) | % Protection (leaves) | VDI[z] (%) | % Protection (vascular tissues) |
|---|---|---|---|---|---|---|
| Control | 56.7±5.7a | – | 33.6±9.9 a | – | 58.6±5.5 a | – |
| Fertilizer | 53.8±2.5 a | 5.1 | 36.7±5.5 a | 0 | 65.0±3.0 a | 0 |
| 0.5% DME | 43.3±5.8 b | 23.7 | 21.8±7.5 b | 35.2 | 42.2±3.9 b | 28.0 |
| 2% DME | 43.3±5.8 b | 23.7 | 15.8±0.8 b | 53.0 | 36.3±2.8 c | 38.1 |
| 5% DME | 28.3±10.4 c | 51.1 | 8.6±4.1 c | 74.4 | 25.2±1.9 d | 57.0 |

[z]DPI, diseased plant index; LWI, leaf wilt index; VDI, vascular discoloration index.
[y]Within columns,values followed by the same letter do not differ significantly ($P$=0.05) according to Duncan's multiple range test.

**Effect of lipid-free or protein-free DME in controlling Verticillium wilt** Data in Table 3 show that 5% DME was as effective as 2% DM in controlling the wilt disease in cv. H552. Elimination of either lipids or proteins from DME did not reduce the controlling efficacy of DME.

**Colonization of vascular system in cotton plants by *V. dahliae*** The colonization of *V. dahliae* in various plant tissues at 4 weeks after inoculation is shown in Table 4. The

TABLE 3. Comparison of Verticillium wilt disease-controlling efficacy in cotton plants between dry mycelium (DM) of *Penicillium chrysogenum* and its various extracts. DM was amended into soil before sowing; DME (water extract of DM) was drenched into soil 13 days after emergence; plants were inoculated with *Verticillium dahliae* 16 days after emergence; data (means ± SD) were recorded 28 days after inoculation

| Treatment | DPI[z] (%) | % Protection (plants) | LWI[z] (%) | % Protection (leaves) | VDI[z] (%) | % Protection (vascular tissues) |
|---|---|---|---|---|---|---|
| Control | 95.0±11.2a | – | 77.5±5.6a | – | 85.0±13.7a | – |
| 2% DM | 65.0±13.5b | 32 | 33.8±10.8b | 55 | 47.9±18.1b | 44 |
| 5% DME | 65.0±13.7b | 32 | 50.0±8.1c | 34 | 41.9±7.5b | 51 |
| 5% Lipid-free DME | 50.0±17.7b | 47 | 30.5±8.0b | 60 | 48.3±18.1b | 43 |
| 5% Protein-free DME | 65.0±28.5b | 32 | 32.0±25.2b | 58 | 54.6±21.7b | 36 |

[z]DPI, diseased plant index; LWI, leaf wilt index; VDI, vascular discoloration index.
[y]Within columns, values followed by the same letter do not differ significantly ($P$=0.05) according to Duncan's multiple range test.

TABLE 4. Effect of dry mycelium (DM) or water extract of DM (DME) of *Penicillium chrysogenum* on colonization of *Verticillium dahliae* (in cfu) in tissues of cotton plants. DM and DME were applied as described in Tables 1 and 2, respectively. Data (means ± SD) were recorded 21 days after inoculation

| Treatment[z] | Colony forming units (cfu $g^{-1}$ fresh weight tissue) × 1000 | | |
|---|---|---|---|
| | Root | Hypocotyls | Epicotyls |
| Control | 367±124a[y] | 214±23a | 85±18a |
| 2% DM | 239±85a | 9±1b | 5±3b |
| 5% DME | 269±59a | 10±9b | 6±2b |

[z] DM was applied to the soil prior to sowing and plants were inoculated with *V. dahliae* 14 days after emergence. DME was applied as a soil drench 11 days after emergence and plants were inoculated 3 days after treatment.
[y] Within columns, values followed by the same letter do not differ significantly ($P$=0.05) according to Duncan's multiple range test.

TABLE 5. Effect of dry mycelium (DM) or water extract of DM (DME) of *Penicillium chrysogenum* on the growth of healthy cotton plants. Data (means ± SD) were recorded 35 days after emergence

| Treatment[z] | Cultivar H552 | | | Cultivar PF15 | | |
|---|---|---|---|---|---|---|
| | Plant height (cm) | Fresh wt/plant (g) | Dry wt/plant (g) | Plant height (cm) | Fresh wt/plant (g) | Dry wt/plant (g) |
| Control | 18.4 ±0.3a[y] | 3.83 ±0.35a | 1.15 ±0.13a | 20.5±1.7a | 4.68±0.52a | 1.45±0.21a |
| 2% DM | 22.8 ±1.5b | 6.23 ±0.36b | 1.95 ±0.16b | 28.7±2.7b | 8.12±0.83b | 2.32±0.26b |
| 5% DME | 20.9 ±1.6c | 5.02 ±0.44c | 1.50 ±0.12c | 26.2±1.8b | 6.84±0.62c | 1.88±0.12c |

[z] DM was applied to the soil before sowing and DME was applied as a soil drench 12 days after emergence.
[y] Within columns,values followed by the same letter do not differ significantly ($P$=0.05) according to Duncan's multiple range test.

number of cfu was drastically reduced in both the hypocotyls and epicotyls of plants treated with either 2% DM or 5% DME, but no significant reduction in cfu occurred in the root tissue, relative to the control plants.

TABLE 6. Effect of dry mycelium (DM) or water extract of DM (DME) of *Penicillium chrysogenum* on the growth of cotton plants inoculated with *Verticillium dahliae*. Data (means ± SD) were recorded 35 days after emergence

| Treatment[z] | Cultivar H552 | | | Cultivar PF15 | | |
|---|---|---|---|---|---|---|
| | Plant height (cm) | Fresh wt/plant (g) | Dry wt/plant (g) | Plant height (cm) | Fresh wt/plant (g) | Dry wt/plant (g) |
| Control | 13.7±1.2a | 2.84±0.03a | 0.85±0.08a | 14.8±1.9a | 3.44±0.16a | 1.06±0.04a |
| 2% DM | 15.6±0.4b | 5.60±0.46b | 1.68±0.12b | 16.7±1.8b | 6.47±0.39b | 1.93±0.12b |
| 5% DME | 15.1±0.8b | 5.02±0.29c | 1.51±0.04c | 16.0±1.1b | 4.72±0.15c | 1.49±0.09c |

[z] DM was applied to the soil prior to sowing and DME was applied as a soil drench 12 days after emergence; plants were inoculated 16 days after emergence (4 days after DME treatment).
[y] Within columns, values followed by the same letter do not differ significantly ($P$=0.05) according to Duncan's multiple range test.

TABLE 7. Percentage increase in plant growth of healthy plants and inoculated plants treated with either 2% dry mycelium (DM) or 5% water extract of DM (DME) of *Penicillium chrysogenum* relative to healthy control plants and inoculated control plants, respectively. Data were obtained from statistical analyses of Tables 5 and 6

| Treatment | H552 | | | PF15 | | |
|---|---|---|---|---|---|---|
| | Plant height | Fresh wt/plant | Dry wt/plant | Plant height | Fresh wt/plant | Dry wt/plant |
| 2% DM | 23.9 | 62.7 | 70.0 | 40.0 | 73.5 | 60.0 |
| 5% DME | 13.6 | 31.1 | 30.4 | 27.8 | 46.2 | 29.7 |
| 2% DM-inoculated | 13.9 | 97.2 | 97.6 | 12.8 | 82.8 | 82.1 |
| 5% DME-inoculated | 10.2 | 76.8 | 77.7 | 8.1 | 37.2 | 40.6 |

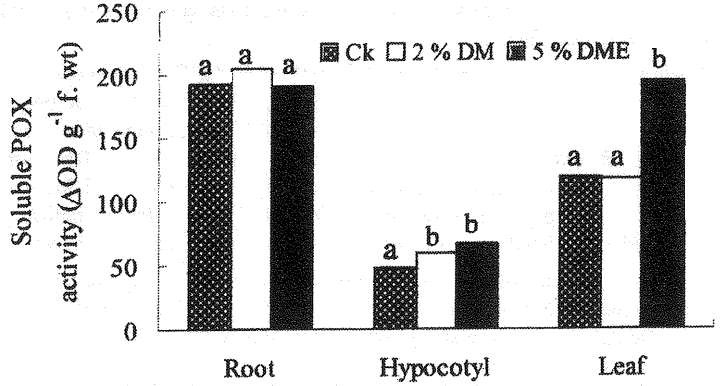


Fig. 1. Soluble peroxidase (POX) activity in cotton plants (cv. H552) treated with 2% (w/w) dry mycelium (DM) or 5% (w/v) water extract of DM (DME). DM was applied to the soil prior to sowing and DME was applied as a soil drench 13 days after emergence. POX activity was measured 16 days after emergence. Values indicated by the same letter do not differ significantly ($P$=0.05) according to Duncan's multiple range test.

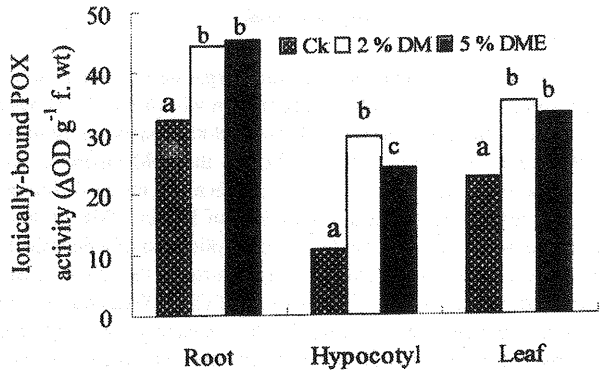


Fig. 2. Ionically-bound peroxidase (POX) activity in cotton plants (cv. H552) treated with 2% (w/w) dry mycelium (DM) or 5% (w/v) water extract of DM (DME). DM was applied to the soil prior to sowing and DME was applied as a soil drench 13 days after emergence. POX activity was measured 16 days after emergence. Values indicated by the same letter do not differ significantly ($P$=0.05) according to Duncan's multiple range test.

**Effect of DM or DME on POX activity in healthy cotton plants** Neither 2% DM nor 5% DME induced a significant change in soluble POX activity in the roots. However, activity in either the hypocotyls or the second leaves was significantly increased in 5% DME-treated plants, but not in 2% DM-treated plants, as compared with water-treated control plants (Fig. 1). Treatments with either 2% DM or 5% DME significantly increased ionically-bound POX activity in roots, hypocotyls and the second leaf, relative to water-

treated plants (Fig. 2). The increase in ionically-bound POX activity in hypocotyls was larger than in other organs (Fig. 2).

***In vitro* effects of DM or DME on mycelial growth of *V. dahliae*** Two concentrations of DM or DME were amended to CZA agar to study the possible fungitoxic activity of DM or DME against *V. dahliae*. Fungal colony diameter recorded at 5 days after inoculation did not differ significantly between control and either DM-amended or DME-amended plates. However, significant increments in colony diameters of DM- or DME-amended plates relative to control plates occurred at 10–20 days after inoculation, indicating that DM and DME had a beneficial effect on growth of this pathogen (data not shown).

**Effect of DM or DME on plant growth** Plant height, fresh and dry weights per plant of healthy cotton plants 35 days after emergence are given in Table 5. Both 2% DM and 5% DME significantly increased plant growth of the two cotton cultivars, relative to the untreated control plants. However, cotton plants treated with 2% DM gained significantly more fresh weight (H552, 63%; PF15, 74%) or dry weight (H552, 70%; PF15, 60%) than those treated with 5% DME (H552, 31%, 46%; PF15, 30%, 30%) (Table 7).

Data in Table 6 show that plant height, fresh weight and dry weight per plant of inoculated plants treated with either 2% DM or 5% DME were significantly greater than of the inoculated control. Moreover, inoculated plants treated with 2% DM gained significantly more fresh weight (H552, 97%; PF15, 83%) or dry weight (H552, 98%; PF15, 82%) than those treated with 5% DME (77%, 37%; 78%, 42%) (Table 7).

## DISCUSSION

A number of chemical compounds and microorganisms were reported to induce resistance against cotton diseases including Verticillium wilt (6,15,24). However, there has been no report on dead fungal biomass which can induce resistance against Verticillium wilt in cotton. Results in the present study indicated that DM amended into the soil or DME drenched to the roots before inoculation significantly reduced disease severity of Verticillium wilt in cotton. Since no inhibitory effect of DM or DME on mycelial growth of this pathogen was observed *in vitro*, and because application of a chemical fertilizer did not reduce disease severity, it is suggested that the control of the wilt disease with DM or DME resulted from induced resistance. Water extract of DM (DME) was as effective as DM (powder) in controlling the disease, implying that the resistance-inducing substances were mostly water-soluble. The fact that elimination of either chloroform-soluble lipophytic materials or proteinous materials (precipitating in cold acetone) from DME did not reduce the efficacy of DME in disease control, suggested that the resistance-inducing substances are probably neither lipids nor proteins (or peptides) contained in DME.

Studies concerning the mechanisms of induced resistance have been performed for almost half a century (23). Such mechanisms depend on the activator, the plant species (20,22) and the pathogens (19,30). In general, defense responses induced by either biotic or abiotic agents include processes of oxygen burst, lignification, accumulation of pathogenesis-related proteins, structural barriers and phytoalexin accumulation (31,32). Among the PR-proteins, chitinase and glucanases possess potential antifungal activities, while POX could be involved in the strengthening of plant cell walls by lignification (34,35).

Previous studies with cotton showed that infection with an aggressive strain of *V.*

*dahliae* induced reinforcement of structural barriers and production of phytoalexins, which played key roles in resistance against Verticillium wilt (3,9). An elicitor of *V. dahliae* increased levels of POX activities, synthesis and deposition of lignin and lignin-like phenolic polymers, thus resulting in resistance against the pathogen (8,11,32). Cotton cultivars with higher and earlier induced levels of POX activity and deposition of lignin possessed enhanced resistance against this disease (32). We report herein that application of DM or DME significantly increased the activity of both soluble and ionically-bound POX in hypocotyls. Significant increments in ionically-bound POX activity were found also in the roots of plants treated with 2% DM or 5% DME. Surprisingly, ionically-bound POX activity in hypocotyls of DM- and DME-treated plants was 1.7-fold and 1.3-fold higher, respectively, than that of the control water-treated plants, and much higher than in other tissues of DM- or DME-treated plants. Since the hypocotyls serve as an essential path for *V. dahliae* to spread systemically to upper tissues, the drastic increase in POX activity in hypocotyls by DM or DME may delay fungal spread due to lignification and thus induce wilt resistance.

No significant difference in colonization of *V. dahliae* in the root was found among DM, DME or water treatments. In contrast, treatment with DM or DME drastically decreased colonization of this pathogen in the hypocotyl and the epicotyl. Similar results were reported with induced resistance in cotton and tomato by MeJA or BABA (24). Since DM and DME caused significantly higher POX activity in hypocotyls than in other tissues, reduction in colonization in hypocotyls might result from cell wall reinforcement and/or production of phytolexins. Phytoalexins were reported to be responsible for the suppression of *V. dahliae* growth in the vascular systems, and were part of the mechanism of induced natural defense responses (24). Hemigossypol was one of the important phytoalexins in cotton active against *V. dahliae* (12).

Many disease control measures including use of fungicides or biological agents could enhance plant growth (15,37), whereas some others, like the use of resistance inducer INA or BTH, were reported to have phytotoxic properties on crops (33). Such responses of growth enhancement were usually attributed to an indirect effect associated with control of plant diseases. However, in some cases, it resulted from the production of growth-regulating substances (37). In the present work, we found that application of either DM or DME significantly promoted growth of healthy cotton plants relative to healthy control plants. Significant increases in plant height, fresh and dry weights were also found in inoculated plants pretreated with DM or DME, compared with water-treated inoculated plants (control). The enhanced growth of healthy plants can be attributed mainly to the nutritional effect of DM and DME. Indeed, it was reported that DM used as an organic fertilizer enhanced growth of sweet corn (13). The growth increase of inoculated plants may be attributed to both nutrients supply and disease control. Nevertheless, the possibility that growth-regulating substances occur in DM or DME can not be excluded.

The DM of *P. chrysogenum* used here was found not only to induce resistance against Verticillium wilt, but also to enhance plant growth. DME was as effective as DM in induction of resistance. Both DM and DME bear the potential to be used in agriculture for plant protection and plant production.

ACKNOWLEDGMENT

This research was supported in part by a Fred and Barbara Kort Sino–Israel Postdoctoral Fellowship to Dr. Hezhong Dong.

## REFERENCES


1. Allen, S.J. (2001) Genetic and induced resistance strategies for controlling Fusarium wilt of cotton. In: *Proc. Second Australasian Soilborne Diseases Symp.* (Victoria, Australia), pp. 59-60.
2. Bejarano-Alcazar, J., Melero-Vara, J.M., Blanco-Lopez, M.A. and Jimenez-Diaz, R.M. (1995) Influence of inoculum density of defoliating and nondefoliating pathotypes of *Verticillium dahliae* on epidemics of Verticillium wilt of cotton in Southern Spain. *Phytopathology* 85:1474-1481.
3. Bell, A.A. and Presley, J.T. (1969) Temperature effects upon resistance and phytoalexin synthesis in cotton inoculated with *Verticillium albo-atrum. Phytopathology* 59:1141-1146.
4. Brock, P.M., Inwood, J.R.B. and Deverall, B.J. (1994) Systemic induced resistance to *Alternaria macrospora* in cotton (*Gossypium hirsutum*). *Australas. Plant Pathol.* 23:81-85.
5. Cohen, Y. (1994) Local and systemic control of *Phytophthora infestans* in tomato plants by DL-3-amino-*n*-butanoic acids. *Phytopathology* 84:55-59.
6. Colson-Hanks, E.S., Allen, S.J. and Deverall, B.J. (2000) Effect of 2,6-dichloroisonicotinic acid or benzothiadiazole on Alternaria leaf spot, bacterial blight and Verticillium wilt in cotton under field conditions. *Australas. Plant Pathol.* 29:170-177.
7. Colson-Hanks, E.S. and Deverall, B.J. (2000) Effect of 2,6-dichloroisonicotinic acid, its formulation materials and benzothiadiazole on systemic resistance to Alternaria leaf spot in cotton. *Plant Pathol.* 49:171-178.
8. Cui, Y.X., Bell, A.A., Joost, O. and Magill, C. (2000) Expression of potential defense response genes in cotton. *Physiol. Mol. Plant Pathol.* 56:25-31.
9. Daayf, F., Nicole, M., Boher, B., Pando, A. and Geiger, J.P. (1997) Early vascular defense reactions of cotton roots infected with a defoliating mutant strain of *Verticillium dahliae. Eur. J. Plant Pathol.* 103:125-136.
10. Dong, H.Z. and Cohen, Y. (2001) Extracts of killed *Penicillium chrysogenum* induce resistance against Fusarium wilt of melon. *Phytoparasitica* 29:421-430.
11. Dubery, I.A. and Slater, V. (1997) Induced defence responses in cotton leaf disks by elicitors from *Verticillium dahliae. Phytochemistry* 44:1429-1434.
12. Eldon, S. and Hillocks, R.J. (1996) The effect of reduced phytoalexin production on the resistance of upland cotton (*Gossypium hirsutum*) to Verticillium and Fusarium wilts. *Ann. Appl. Biol.* 129:217-225.
13. Gao, J., Ben-Daniel, B.H. and Cohen, Y. (2001) Organic fertilizers of microbial origin enhance growth and reduce infection by *Fusarium moniliforme* of sweet corn. *Phytoparasitica* 29:268 (abstr.).
14. Hampton, R.E., Wullschleger, S.D. and Oosterhuis, D.M. (1990) Impact of Verticillium wilt on net photosynthesis, respiration and photorespiration in field grown cotton (*Gossypium hirsutum* L.). *Physiol. Mol. Plant Pathol.* 37:271-280.
15. Hanson, L.E. (2000) Reduction of Verticillium wilt symptoms in cotton following seed treatment with *Trichoderma virens. J. Cotton Sci.* 4:224-231.
16. Haselwandter, K. (1997) Soil micro-organisms, mycorrhiza, and restoration ecology. *in:* Urbanska, K.M., Webb, N.R. and Edwards, P.J. [Eds.] Restoration Ecology and Sustainable Development. Cambridge University Press, Cambridge, UK. pp. 65-80.
17. Howell, C.R., Hanson, L.E., Stipanovic, R.D. and Puckhaber, L.S. (2000) Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens. Phytopathology* 90:248-252.
18. Jackson, A.J., Walters, D.R. and Marshall, G. (1994) Evaluation of *Penicillium chrysogenum* and its antifungal extracts as potential biological control agents against *Botrytis fabae* on faba beans. *Mycol. Res.* 98:1117-1126.
19. Jakab, G., Cottier, V., Toquin, V., Rigoli, G., Zimmerli, L., Metraux, J.-P. *et al.* (2001) $\beta$-Aminobutyric acid-induced resistance in plants. *Eur. J. Plant Pathol.* 107:29-37.
20. Jeun, Y.C., Siegrist, J. and Buchenauer, H. (2000) Biochemical and cytological studies on mechanisms of systemically induced resistance to *Phytophthora infestans* in tomato plants. *J. Phytopathol.* 148:129-140.
21. Karban, R., Adamchak, R. and Schnathorst, W.C. (1987) Induced resistance and interspecific competition between spider mites and a vascular wilt fungus. *Science* 35:678-680.
22. Kessmann, H., Staub, T., Hofmann, C., Maetzke, T., Herzog, J., Ward, E. *et al.* (1994) Induction of systemic acquired resistance in plants by chemicals. *Annu. Rev. Phytopathol.* 32:439-459.
23. Kuć, J. (2001) Concepts and direction of induced systemic resistance in plants and its application. *Eur. J. Plant Pathol.* 107:7-12.
24. Li, J., Zingen-Sell, I. and Buchenauer, H. (1996) Induction of resistance of cotton plants to Verticillium wilt and of tomato plants to Fusarium wilt by 3-aminobutyric acid and methyl jasmonate. *J. Plant Dis. Prot.* 103:288-299.

25. Oka, Y., Cohen, Y. and Spiegel, Y. (1999) Local and systemic induced resistance to the root-knot nematode in tomato by DL-$\beta$-amino-*n*-butyric acid. *Phytopathology* 89:1138-1143.
26. Oostendorp, M., Kunz, W., Dietrich, B. and Staub, T. (2001) Induced disease resistance in plants by chemicals. *Eur. J. Plant Pathol.* 107:19-28.
27. Paplomatas, E.J., Bassett, D.M,. Broome, J.C. and Devay, J.E. (1992) Incidence of Verticillium wilt and yield losses of cotton cultivars (*Gossypium hirsutum*) based on soil inoculum density of *Verticillium dahliae*. *Phytopathology* 82:1417-1420.
28. Schnathorst, W.C. (1981) Life cycle and epidemiology of Verticillium. *in:* Mace, M.E., Bell, A.A. and Beckman, C.H. [Eds.] Fungal Wilt Disease of Plants. Academic Press, New York, NY. pp. 81-111.
29. Schnathorst, W.C. and Mathre, D.E. (1966) Cross protection in cotton with strains of *Verticillium albo-atrum*. *Phytopathology* 56:1024-1029.
30. Siegrist, J., Glenewinkel, D., Kolle, C. and Schmidtke, M. (1997) Chemically induced resistance in green bean against bacterial and fungal pathogens. *J. Plant Dis. Prot.* 104:599-610.
31. Siegrist, J., Orober, M. and Buchenauer, H. (2000) Beta-aminobutyric acid-mediated enhancement of resistance in tobacco to tobacco mosaic virus depends on the accumulation of salicylic acid. *Physiol. Mol. Plant Pathol.* 56:95-106.
32. Smit, F. and Dubery, I.A. (1997) Cell wall reinforcement in cotton hypocotyls in response to a *Verticillium dahliae* elicitor. *Phytochemistry* 44:811-815.
33. Sticher, L., Mauch-Mani, B. and Metraux, J.P. (1997) Systemic acquired resistance. *Annu. Rev. Phytopathol.* 35:235-270.
34. van Loon, L.C. (1997) Induced resistance in plants and the role of pathogenesis-related proteins. *Eur. J. Plant Pathol.* 103:753-765.
35. van Loon, L.C., Pierpoint, W.S., Boller, T. and Conejero, V. (1994) Recommendations for naming plant-related proteins. *Plant Mol. Biol. Rep.* 12:245-264.
36. Wilhelm, S., Sagen, J. and Tietz, H. (1985) Phenotype modification in cotton for control of Verticillium wilt through dense plant population culture. *Plant Dis.* 69:283-288.
37. Windham, M.T., Elad, Y. and Baker, R. (1986) A mechanism for increased plant growth induced by *Trichoderma* spp. *Phytopathology* 76:518-521.