

## Degradation of Fungal Cell Walls by Lytic Enzymes of *Trichoderma harzianum*

By ALEX SIVAN\*† AND ILAN CHET

Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem,  
Faculty of Agriculture, Rehovot, 76100, Israel

(Received 18 May 1988; revised 12 October 1988; accepted 16 November 1988)

---

In *in vitro* tests, two strains of *Trichoderma harzianum* failed to parasitize colonies of *Fusarium oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f. sp. *melonis*. However, these strains were strongly mycoparasitic on *Rhizoctonia solani* and *Pythium aphanidermatum*. When grown in liquid cultures containing laminarin, chitin or fungal cell walls as sole carbon sources, both strains of *T. harzianum* released 1,3- $\beta$ -glucanase and chitinase into the medium. Higher levels of these enzymes were induced in strain T-203 than in T-35 by hyphal cell walls of *F. oxysporum*. When the lytic enzymes produced by T-35 were incubated with hyphal cell walls of the test fungi, more glucose and *N*-acetyl-D-glucosamine was released from cell walls of *R. solani* and *Sclerotium rolfisii* than from those of *F. oxysporum*. Treatment of *F. oxysporum* cell walls with 2 M-NaOH, protease or trypsin prior to their incubation with the lytic enzymes of *T. harzianum* significantly increased the release of glucose and *N*-acetyl-D-glucosamine. The effect of these treatments on *R. solani* and *S. rolfisii* cell walls was much lower. These results suggest that proteins in the cell walls of *F. oxysporum* may make these walls more resistant than those of *R. solani* or *S. rolfisii* to degradation by extracellular enzymes of *T. harzianum*.

---

### INTRODUCTION

The direct mycoparasitic activity of *Trichoderma* spp. is one of the major mechanisms proposed to explain their antagonistic activity against soil-borne plant-pathogenic fungi (Dennis & Webster, 1971; Elad *et al.*, 1982; Lynch, 1987; Ridout *et al.*, 1986). The lytic activity of fungal, as well as of bacterial, antagonists is mainly due to the lytic enzymes 1,3- $\beta$ -glucanase and chitinase (Mitchell & Alexander, 1963; Henis & Chet, 1975). 1,3- $\beta$ -Glucanase is a semi-constitutive enzyme (Bull & Chesters, 1966) which may be induced by several inducers such as laminarin, starch, xylose, mannitol and glycerol (Reese & Mandels, 1959). However, in the presence of laminarin the excretion of this enzyme increases (Elad *et al.*, 1982). Chitinase is an inducible enzyme excreted by many micro-organisms in cultures containing chitin or its oligomers as sole carbon source (Monreal & Reese, 1969).

Different strains of *Trichoderma harzianum* effective in controlling *Rhizoctonia solani* produced 1,3- $\beta$ -glucanase and chitinase in cultures containing cell walls of this pathogen as a sole carbon source (Elad *et al.*, 1982; Hadar *et al.*, 1979; Ridout *et al.*, 1986). Similarly, Tokimoto (1982) reported the production of these enzymes in dual cultures of *T. harzianum* and *Lentinus edodes*. It has been suggested that the lytic activity of several strains of *T. harzianum* on cell walls of *Sclerotium rolfisii*, *Rhizoctonia solani* and *Pythium aphanidermatum* can be correlated with the degree of biological control of those pathogens *in vivo* (Artigues & Davet, 1984; Elad *et al.*, 1982).

---

† Present address: Department of Horticultural Sciences, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, USA.

Abbreviations: SM, synthetic medium; YM, yeast-extract/glucose medium; PDA, potato dextrose agar; GlcNAc, *N*-acetyl-D-glucosamine.

The objective of the present study was to evaluate the possible role of mycoparasitism in the biological control of *Fusarium oxysporum* obtained with a new strain of *T. harzianum*. This strain is one of the very few strains effective in controlling this pathogen (Sivan & Chet, 1986; Sivan *et al.*, 1987).

#### METHODS

**Fungal strains and growth media.** *Trichoderma harzianum* strains were cultured at 30 °C on a synthetic medium (SM; Okon *et al.*, 1973) containing (g per litre of distilled water): glucose, 15; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.2; KH<sub>2</sub>PO<sub>4</sub>, 0.9; KCl, 0.2; NH<sub>4</sub>NO<sub>3</sub>, 1.0; Fe<sup>2+</sup>, 0.002; Zn<sup>2+</sup>, 0.002; agar, 20 (in solid medium). The mycolytic activity of the biocontrol agent (*T. harzianum* strain T-35) against *Fusarium oxysporum* was compared with that of another *T. harzianum* strain (T-203) isolated by Elad *et al.* (1980) and reported as an effective mycoparasite of *Rhizoctonia solani* Kuhn and *Sclerotium rolfsii* Sacc. (Elad *et al.*, 1982). *F. oxysporum* f. sp. *melonis* Snyder & Hans. (*F. o. melonis*) and *F. oxysporum* f. sp. *vasinfectum* (Atk.) Snyder & Hans. (*F. o. vasinfectum*) were isolated from infected melon and cotton plants, respectively (Sivan & Chet, 1986) and cultured at 27 °C on a yeast extract/glucose medium (YM) containing (g per litre of distilled water): yeast extract (Difco), 5; peptone (Difco), 5; glucose, 10; agar, 20 (in solid medium). *R. solani*, *S. rolfsii* and *Pythium aphanidermatum* were grown on SM at 30 °C.

**Dual culture tests.** Mycelial disks (5 mm in diameter) of *F. o. melonis*, *F. o. vasinfectum*, *R. solani* or *P. aphanidermatum* were placed on one edge of a Petri dish containing PDA, while mycelial disks of *T. harzianum* were placed on the opposite side of the plate. Because of the lower growth rate of *F. oxysporum*, in each test where this fungus was used, the inoculation with mycelial disks of *T. harzianum* was performed 72 h after that of *F. oxysporum*; otherwise the inoculation with *T. harzianum* was performed simultaneously with that of the test fungi. After the desired incubation time, at 27 °C, the overgrowth of colonies of the test fungi by the antagonist was determined.

Survival of mycelium of *F. oxysporum* and *S. rolfsii* was determined after exposure to conidia of *T. harzianum*. Mycelial disks (5 mm in diameter) were removed from 72-h-old cultures of the tested fungi on water agar (20 g agar per litre of distilled water). Disks were soaked for 30 s in a conidial suspension of *T. harzianum* (10<sup>6</sup> conidia ml<sup>-1</sup>) and incubated in Petri dishes, each containing four layers of wet Whatman no. 1 filter paper. Mycelial disks treated with autoclaved distilled water and incubated as described above served as controls. After the desired incubation time, mycelial disks of *F. oxysporum* were transferred to a Fusarium-selective medium (Nash & Snyder, 1962) containing 1 mg methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (Benomyl) l<sup>-1</sup>, in order to inhibit growth of *T. harzianum* (Elad & Chet, 1983). Similarly, mycelial disks of *S. rolfsii* were transferred to SM plates containing the same concentration of Benomyl. The results were expressed as the percentage of the mycelial disks from where the test fungus grew.

**Preparation of hyphal cell walls.** Cell walls were obtained from *F. o. melonis*, *F. o. vasinfectum*, *R. solani* and *S. rolfsii* after culture at 27 °C for 5 d in 50 ml liquid YM. Flasks with cultures of *R. solani* or *S. rolfsii* were shaken in a rotary shaker at 120 r.p.m., while those of *F. oxysporum* were incubated without shaking, to reduce conidiation. After incubation, mycelia were thoroughly washed with distilled autoclaved water and homogenized on ice, with an Ultra Turax homogenizer (Ika-Werk, W. Germany) for 5 min at the highest speed. Mycelial suspension was centrifuged at 30000 g for 20 min at 4 °C. The pellet was resuspended in distilled water and sonicated on ice three times for 5 min each using a Heat Systems-Ultrasonics sonicator at full amplitude. The suspension was centrifuged again at 800 g for 10 min at 4 °C to precipitate the coarse particles. The supernatant containing the remaining fine particles was washed by centrifugation and homogenized intermittently until no residual glucose, protein or amino acids could be detected in the supernatant, as determined with glucose oxidase (Sigma), Coomassie brilliant blue (Sedmak & Grossberg, 1977) or ninhydrin (Cocking & Yemm, 1954) reagents, respectively. The precipitated walls were then deep-frozen, lyophilized and kept as powder in a sealed container until use (Chet & Huttermann, 1980).

**Induction of extracellular lytic enzymes in *T. harzianum*.** Erlenmeyer flasks (250 ml) each containing 50 ml liquid SM were inoculated with 1 ml of a conidial suspension (5 × 10<sup>7</sup> conidia ml<sup>-1</sup>) of *T. harzianum*. The glucose in the medium was substituted with one of the following carbon sources (each at 2 mg ml<sup>-1</sup>): laminarin (Sigma), colloidal chitin (prepared according to Rodriguez-Kabana *et al.*, 1983), or one of the fungal cell wall preparations. Cultures were incubated at 28 °C in a rotary shaker at 120 r.p.m. for the desired time, then centrifuged at 15000 g at 4 °C for 10 min. The supernatant was dialysed against distilled water at 4 °C for 24 h to eliminate residual glucose or *N*-acetyl-D-glucosamine (GlcNAc). The dialysate was lyophilized or tested directly for enzyme activity. Filtrates from chitin cultures also contained traces of 1,3-β-glucanase, but those from laminarin cultures contained no detectable chitinase.

**Enzyme assays.** The activity of extracellular lytic enzymes was tested according to Elad *et al.* (1982). 1,3-β-Glucanase (exo-1,3-β-D-glucosidase, EC 3.2.1.58) was assayed by following the release of free glucose from laminarin using the glucose oxidase reagent. Specific activity was expressed as μmol glucose h<sup>-1</sup> (mg protein)<sup>-1</sup>. The reaction mixture, containing 1 ml crude 1,3-β-glucanase, 1 ml 0.1 M-citrate buffer (pH 5.1) and 1.6 mg soluble

laminarin, was incubated at 38 °C for 1 h. The reaction was stopped by placing the reaction mixture in boiling water.

Chitinase (1,4- $\beta$ -poly-*N*-acetyl-D-glucosaminidase, EC 3.2.1.14) was assayed by following the release of GlcNAc from colloidal chitin (Reissig *et al.*, 1959). Specific activity was expressed as  $\mu\text{mol GlcNAc h}^{-1}$  ( $\text{mg protein}^{-1}$ ). The reaction mixture, containing 1 ml crude chitinase, 1 ml 0.1 M-citrate buffer (pH 5.1) and 1.6 mg colloidal chitin, was incubated at 38 °C for 2 h and the reaction then stopped by boiling.

The release of monomers from fungal cell walls was also tested using lyophilized crude enzymes from cultures containing chitin or laminarin as sole carbon sources and designated chitinase or 1,3- $\beta$ -glucanase, respectively. The reaction mixture (2 ml), containing 1.5 mg lyophilized enzyme and 1.6 mg cell walls  $\text{ml}^{-1}$  was incubated at 38 °C for 24 h. Each test tube was amended with 10  $\mu\text{l}$  methylbenzene (toluene) to prevent contamination.

The activity of these enzymes on a living mycelium was tested by using mycelial mats of *F. o. melonis* grown in liquid YM for 96 h. Each mycelial mat was washed with sterile citrate buffer (pH 5.1) and transferred to a 250 ml Erlenmeyer flask containing 25 ml of the same buffer and lyophilized enzyme (1  $\text{mg ml}^{-1}$ ) obtained from culture filtrates of *T. harzianum* grown on laminarin or chitin as sole carbon source.

**Modification of hyphal cell walls.** In some experiments, cell walls were treated prior to application of the lytic enzymes. In all these treatments, 40 mg of cell walls were suspended and shaken for 1 h at 50 r.p.m. in 60 ml of (a) 2 M-NaOH at 25 °C, (b) chloroform/methanol (2:1, v/v) at 25 °C, (c) protease (Sigma type XXV) (300  $\mu\text{g ml}^{-1}$  in 0.1 M-phosphate buffer pH 7.0) at 37 °C or (d) trypsin (100  $\mu\text{g ml}^{-1}$  in 0.1 M-phosphate buffer pH 7.0) at 37 °C. The proteolytic activity was stopped by boiling for 10 min at 100 °C.

After each treatment the agent was removed and the hyphal walls were thoroughly washed by centrifugation (15000 g at 4 °C), suspended in flasks containing 20 ml 0.1 M-citrate buffer (pH 5.1) amended with lyophilized crude 1,3- $\beta$ -glucanase or chitinase, and incubated for 24 h at 38 °C.

**Reproducibility.** In all experiments, three replicates of each treatment were used. All experiments were performed at least twice.

## RESULTS

### *In vitro* tests

Both strains of *T. harzianum* failed to parasitize colonies of *F. o. melonis* and *F. o. vasinfectum* even after 160 h incubation. However, when cultured on PDA plates with *R. solani*, *P. aphanidermatum* or *S. rolfsii*, *T. harzianum* strain T-35 overgrew the test fungal colonies. In dual culture plates of T-35 and *P. aphanidermatum*, after 120 h incubation the antagonist covered most of the test colony, while the rate of colonization of *R. solani* and especially of *S. rolfsii* was lower (Table 1). Similar results were obtained when *T. harzianum* T-203 – the mycoparasite of *R. solani* and *S. rolfsii* (Elad *et al.*, 1980) – served as the antagonist.

Dual culture tests performed with other media – SM, YM and Malt-extract agar (Sigma) – showed similar results.

After treatment of disks of *F. o. melonis* and *F. o. vasinfectum* with conidia of T-35 or T-203 and incubation for up to 16 d, hyphae survived in 100 and 75% of the disks, respectively. However, when *S. rolfsii* was subjected to the same treatment for 7 d, no hyphae survived (Fig. 1).

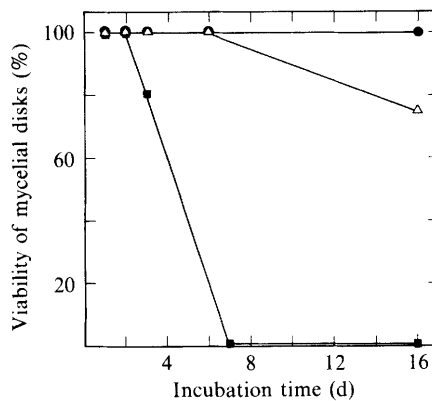


Fig. 1. Survival of mycelial disks of *F. o. vasinfectum* (●), *F. o. melonis* (△) and *S. rolfsii* (■) after treatment with conidia of *T. harzianum* T-35 ( $10^6$  conidia  $\text{ml}^{-1}$ ).

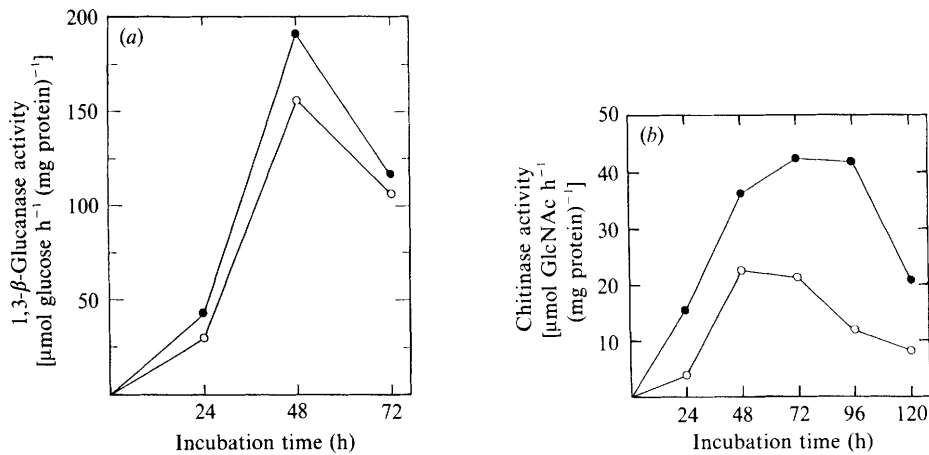


Fig. 2. Specific activity of (a) 1,3- $\beta$ -glucanase and (b) chitinase produced by *T. harzianum* T-35 (○) and T-203 (●) during growth in liquid SM containing cell walls of *F. o. melonis* (2 mg ml<sup>-1</sup>) as sole carbon source.

Table 1. *In vitro* mycoparasitism of test fungi by *T. harzianum* T-35

The data represent the average extent of growth of hyphae of T-35 over mycelium of the test fungus. *F. o. melonis* and *F. o. vasinfectum* were not overgrown by *T. harzianum*, even after 160 h incubation.

Test fungus	Overgrowth of fungal colonies by <i>T. harzianum</i> (mm)		
	Incubation time (h):		
	72	96	120
<i>S. rolfsii</i>	0	2.1	4.5
<i>R. solani</i>	15.1	19.2	22.5
<i>P. aphanidermatum</i>	27.0	32.5	38.1

#### Activity of cell-wall-degrading enzymes in *T. harzianum*

When *T. harzianum* T-35 and T-203 were cultured in liquid medium containing laminarin as sole carbon source, both strains produced high levels of 1,3- $\beta$ -glucanase [165.5 and 210.3  $\mu\text{mol glucose h}^{-1} (\text{mg protein})^{-1}$  after 48 h incubation, respectively]. After growth on chitin as sole carbon source for 48 h the level of chitinase released to the medium was similar in both strains [5.3 and 5.5  $\mu\text{mol GlcNAc h}^{-1} (\text{mg protein})^{-1}$  in T-35 and T-203, respectively].

When the *T. harzianum* strains were grown in media amended with hyphal cell walls of *F. o. melonis* as sole carbon source (Fig. 2a, b), the levels of 1,3- $\beta$ -glucanase and chitinase secreted by T-203 were higher than those produced by T-35. In both strains, maximal induction of 1,3- $\beta$ -glucanase was obtained after 48 h incubation (Fig. 2a). However, the maximal level of chitinase was obtained after 72 h in T-203, but after 48 h in T-35 (Fig. 2b).

The specific activities of the extracellular lytic enzymes of *T. harzianum* T-35 were also tested using cell walls of the four test fungi as the substrate (Fig. 3). The lytic activity of 1,3- $\beta$ -glucanase was higher when incubated with cell walls of *S. rolfsii* or *R. solani* than with those of *F. o. vasinfectum* and *F. o. melonis*. Similarly, when incubated with chitinase, the release of GlcNAc from cell walls of *S. rolfsii* was the highest amongst the tested hyphal walls. On the other hand, there was little or no difference between the specific activity of chitinase after incubation with cell walls of *R. solani* and the fusaria (Fig. 3).

The release of monomers from a live mycelium of *F. o. melonis* after treatment with the crude lytic enzymes of T-35 was minimal (Fig. 4).

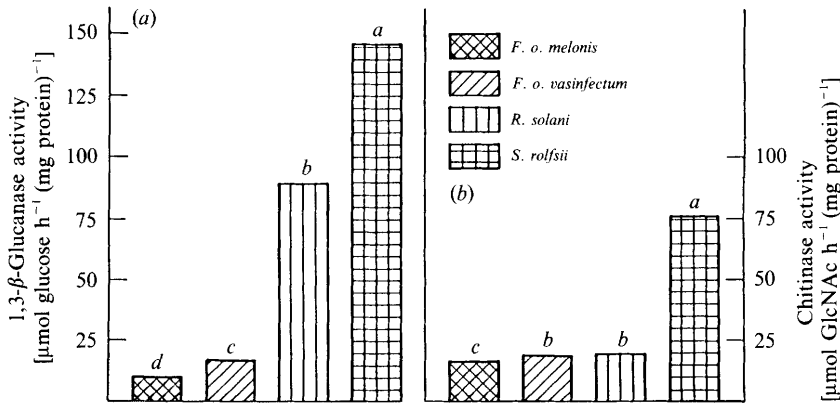


Fig. 3. Specific activity of (a) 1,3- $\beta$ -glucanase and (b) chitinase of *T. harzianum* T-35 incubated with cell walls ( $1 \text{ mg ml}^{-1}$ ) of the four fungi indicated. Columns marked by the same letter are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

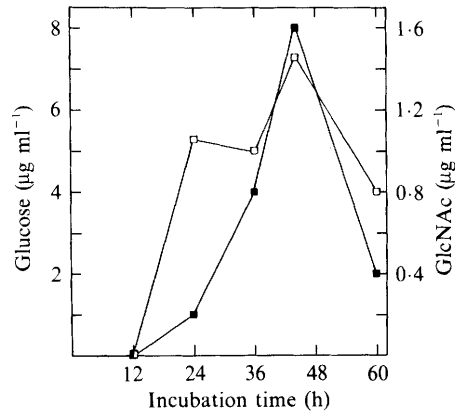


Fig. 4. Release of glucose and GlcNAc from a live mycelium of *F. oxysporum* f. sp. *melonis* by, respectively, 1,3- $\beta$ -glucanase ( $\square$ ) and chitinase ( $\blacksquare$ ) of *T. harzianum* (T-35). The lytic enzymes were incubated with mycelial mats of *F. oxysporum* in a reaction mixture containing  $1 \text{ mg}$  lyophilized crude enzyme  $\text{ml}^{-1}$  and one 96-h-old mycelial mat in  $25 \text{ ml}$  citrate buffer ( $\text{pH } 5.1$ ) amended with  $0.5 \text{ ml}$  toluene.

The possible interference of a fusarial cell wall moiety in the mycoparasitic activity of *T. harzianum* T-35 was tested by treating cell walls with alkali or organic solvent prior to incubation with the lytic enzymes (Table 2). 1,3- $\beta$ -Glucanase incubated with NaOH-treated cell walls of both fusaria released more glucose (up to 13.5-fold) compared with incubation with untreated walls. Similarly, an improvement in chitinase activity was found with the NaOH-treated *Fusarium* walls. In contrast, NaOH treatment had only a slight effect on the susceptibility of walls of *R. solani* and *S. rolfsii* to attack by the two lytic enzymes (Table 2). Treatment with chloroform/methanol did not increase the release of glucose or GlcNAc from walls of either *Fusarium* strain (Table 2).

The hypothesis that a protein or a protein-like constituent(s) is involved in the resistance of fusarial cell walls to lytic enzymes was tested using proteolytic enzymes (Table 3). Protease or trypsin treatment of walls of *F. o. melonis* or *F. o. vasinfectum* before incubation with 1,3- $\beta$ -glucanase or chitinase increased the release of monomers, as compared with nontreated walls. However, proteolytic treatments had little effect on cell walls of *R. solani* and *S. rolfsii* (Table 3).

Table 2. *Effect of NaOH and chloroform/methanol treatments on the release of monomers from cell walls of F. oxysporum, R. solani and S. rolfsii by 1,3-β-glucanase and chitinase of T. harzianum T-35*

Cell walls of	Release of monomers ( $\mu\text{g ml}^{-1}$ ) after treatment:					
	None		2 M-NaOH		Chloroform/ methanol (2:1)	
	Glucose	GlcNAc	Glucose	GlcNAc	Glucose	GlcNAc
<i>F. o. melonis</i>	14.5	42.5	152.5	241.4	6.0	44.0
<i>F. o. vasinfectum</i>	12.0	32.3	162.5	246.1	9.0	30.5
<i>R. solani</i>	76.0	22.0	102.5	74.0	74.0	31.2
<i>S. rolfsii</i>	63.0	193.0	82.0	125.5	73.0	250.8

Table 3. *Effect of proteolytic enzyme treatments on the release of monomers from cell walls of F. oxysporum, R. solani and S. rolfsii by 1,3-β-glucanase and chitinase of T. harzianum T-35*

Cell walls of	Release of monomers ( $\mu\text{g ml}^{-1}$ ) after treatment:					
	None		Protease		Trypsin	
	Glucose	GlcNAc	Glucose	GlcNAc	Glucose	GlcNAc
<i>F. o. melonis</i>	11.8	41.2	37.2	59.6	44.1	79.8
<i>F. o. vasinfectum</i>	7.4	42.0	44.7	78.8	48.9	86.0
<i>R. solani</i>	88.2	43.2	81.9	30.2	86.3	41.0
<i>S. rolfsii</i>	122.1	153.2	109.7	121.0	131.5	152.8

#### DISCUSSION

The mycoparasitic potential of *Trichoderma* spp. is well established (Dennis & Webster, 1971; Elad *et al.*, 1982; Lynch, 1987). This trait has often been utilized as a means of *in vitro* screening for biocontrol candidates (Elad *et al.*, 1980; Hadar *et al.*, 1979). In the present study, using the same dual culture technique, neither of the tested *T. harzianum* strains showed a significant mycoparasitic interaction with *Fusarium oxysporum*. T-35, however, parasitized mycelium of *Rhizoctonia solani*, *Pythium aphanidermatum* and *Sclerotium rolfsii*. Moreover, the survival of mycelium of *F. oxysporum* treated with conidia of T-35 was markedly higher than that of *S. rolfsii*. Similar results were also obtained with T-203, a mycoparasite of *R. solani* and *S. rolfsii* (Elad *et al.*, 1982). This was the first indication of the higher resistance of *F. oxysporum* to lysis. Lynch (1987) demonstrated the overgrowth of *Fusarium* spp. by two *Trichoderma* strains. Thus, it appears that the potential of *Trichoderma* spp. to parasitize *Fusarium* is strain dependent.

*T. harzianum* is, however, an effective biocontrol agent of *F. oxysporum* on several crops (Sivan & Chet, 1986, 1987). The lack of mycoparasitic interaction between *T. harzianum* and *F. oxysporum* indicates that this mechanism is unimportant in this specific system. Therefore, *in vitro* dual culture tests appear not to be a sufficient screen for effective biocontrol agents against *F. oxysporum*.

Two hypotheses for the ineffectiveness of mycoparasitism against *F. oxysporum* were evaluated: (1) lytic enzymes of *T. harzianum* (e.g. 1,3-β-glucanase and chitinase) are not excreted, or (2) lytic enzymes are produced and released but the cell walls of *F. oxysporum* are more resistant to lysis than other fungal cell walls.

To test the first hypothesis we compared the induction and activity of lytic enzymes of *T. harzianum* T-35 with those of the mycoparasite T-203. When grown in liquid medium containing laminarin or chitin as sole carbon source both strains secreted similar amounts of both enzymes. On the other hand, when the strains were cultured on cell walls of *F. oxysporum* as sole carbon source the release of chitinase (but not 1,3-β-glucanase) was 90% higher from T-203 than from T-35. Similarly, chitinase produced by T-203 released more GlcNAc from cell walls of *F. o.*

*melonis* than did the chitinase of T-35. However, neither T-35 nor T-203 parasitizes fusaria; and only T-35 is an effective biocontrol agent of fusarial wilt diseases. Thus, the level of lytic enzyme production is unrelated to either mycoparasitism or biocontrol capability. Similar enzyme preparations did, however, degrade hyphal walls of *S. rolfisii* and *R. solani*, which suggests that fusarial cell walls are more resistant to lysis (hypothesis 2). These results were confirmed by the inability of T-35 to degrade cell walls of live mycelium of *F. oxysporum*. Similarly, the mycoparasite *Pythium nunn* was unable to degrade live mycelium of *F. oxysporum* f. sp. *cucumerinum* (Elad *et al.*, 1985).

Thus our second hypothesis, that cell walls of *F. oxysporum* are more resistant to mycoparasitism, is probably correct. Treatment of hyphal walls of *F. oxysporum* with NaOH or proteolytic enzymes increased their susceptibility to lysis by chitinase and 1,3- $\beta$ -glucanase of *T. harzianum* T-35. Pretreatment of the same hyphal walls with an organic solvent had almost no effect on their lysis. This suggests that fusarial cell walls contain a proteinaceous interfering substance. However, neither the alkali nor the proteolytic pretreatment gave such an effect with cell walls of *R. solani* or *S. rolfisii*. Elad *et al.* (1985) similarly found that lytic enzymes produced by *Pythium nunn* also acted more effectively on trypsin-treated walls of *F. oxysporum* f. sp. *cucumerinum* than on non-treated walls. They postulated the presence of a mucilaginous layer on hyphae of fusaria that protects cell walls against degradation.

We have recently found that *Fusarium oxysporum* cell walls contain more protein than walls of other fungi (unpublished results). Other fusaria also have high (7–28%) protein contents (Barran *et al.*, 1975; Laborda *et al.*, 1974; Schneider *et al.*, 1977). Schneider *et al.* (1977) suggested that the very high protein content of chlamydospores of *F. sulfureum* (21%) may be responsible for their ability to resist lysis in soil.

Our present study suggests that the lack of mycoparasitic interaction between *T. harzianum* (T-35) and *F. oxysporum* may be a result of an outer layer of protein in the hyphal walls of the latter, thus increasing their resistance to lysis. The significant biological control of *F. oxysporum* obtained by this strain (Sivan & Chet, 1986; Sivan *et al.*, 1987) may be due to other mechanisms such as competition (Sivan & Chet, 1989) or antibiosis.

This study was supported in part by the National Council of Research and Development (Israel) and the GSF Munchen, West Germany.

The authors gratefully acknowledge the critical review of the manuscript by Dr G. E. Harman, NY State Agricultural Experiment Station Geneva, NY, USA, and the technical assistance of Mr J. Inbar.

#### REFERENCES

- ARTIGUES, M. & DAVET, P. (1984). Activités (1–3) glucanase et chitinase de quelques champignons, en relation avec leur aptitude à détruire les sclérotés de *Corticium rolfisii* dans la terre stérile. *Soil Biology and Biochemistry* **16**, 527–538.
- BARRAN, L. R., SCHNEIDER, E. F., WOOD, P. J., MADHOSINGH, C. & MILLER, W. R. (1975). Cell wall of *Fusarium sulphureum*. I. Chemical composition of the hyphal wall. *Biochimica et biophysica acta* **392**, 148–158.
- BULL, A. T. & CHESTERS, C. G. C. (1966). The biochemistry of laminarin and the nature of laminarinase. *Advances in Enzymology* **28**, 325–364.
- CHET, I. & HUTTERMANN, A. (1980). Chemical composition of hyphal walls of *Fomes annosus*. *European Journal of Forest Pathology* **10**, 65–70.
- COCKING, E. C. & YEMM, E. W. (1954). Estimation of amino acids by ninhydrin. *Biochemical Journal* **58**, xii.
- DENNIS, C. & WEBSTER, J. (1971). Antagonistic properties of species-groups of *Trichoderma*. III. Hyphal interaction. *Transactions of the British Mycological Society* **57**, 363–369.
- ELAD, Y. & CHET, I. (1983). Improved selective media for isolation of *Trichoderma* spp. or *Fusarium* spp. *Phytoparasitica* **11**, 55–58.
- ELAD, Y., CHET, I. & KATAN, J. (1980). *Trichoderma harzianum*: a biocontrol agent effective against *Sclerotium rolfisii* and *Rhizoctonia solani*. *Phytopathology* **70**, 119–121.
- ELAD, Y., CHET, I. & HENIS, Y. (1982). Degradation of plant pathogenic fungi by *Trichoderma harzianum*. *Canadian Journal of Microbiology* **28**, 719–725.
- ELAD, Y., LIFSHITZ, R. & BAKER, R. (1985). Enzymatic activity of the mycoparasite *Pythium nunn* during interaction with host and non-host fungi. *Physiological Plant Pathology* **27**, 131–148.
- HADAR, Y., CHET, I. & HENIS, Y. (1979). Biological control of *Rhizoctonia solani* damping-off with wheat bran culture of *Trichoderma harzianum*. *Phytopathology* **69**, 64–68.
- HENIS, Y. & CHET, I. (1975). Microbiological control of plant pathogens. *Advances in Applied Microbiology* **19**, 85–111.
- LABORDA, F., GARCIA-ACHA, I., URUBURU, F. & VILLANUEVA, J. R. (1974). Structure of the conidial

- wall of *Fusarium culmorum*. *Transactions of the British Mycological Society* **62**, 557-566.
- LYNCH, J. M. (1987). In vitro identification of *Trichoderma harzianum* as a potential antagonist of plant pathogens. *Current Microbiology* **16**, 49-53.
- MITCHELL, R. & ALEXANDER, M. (1963). Lysis of soil fungi by bacteria. *Canadian Journal of Microbiology* **15**, 689-696.
- MONREAL, J. & REESE, E. T. (1969). The chitinase of *Serratia marcescens*. *Canadian Journal of Microbiology* **15**, 689-696.
- NASH, S. M. & SNYDER, W. C. (1962). Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. *Phytopathology* **52**, 567-572.
- OKON, Y., CHET, I. & HENIS, Y. (1973). Effect of lactose, ethanol and cycloheximide on the translocation pattern of radioactive compounds and on sclerotium formation in *Sclerotium rolfsii*. *Journal of General Microbiology* **74**, 251-258.
- REESE, E. T. & MANDELS, M. (1959).  $\beta$ -1,3-Glucanase in fungi. *Canadian Journal of Microbiology* **5**, 173-185.
- REISSIG, J. L., STROMINGER, J. L. & LOLOIR, L. F. (1959). A modified colorimetric method for the estimation of *N*-acetyl sugars. *Journal of Biological Chemistry* **217**, 959-962.
- RIDOUT, C. J., COLEY-SMITH, J. R. & LYNCH, J. M. (1986). Enzyme activity and electrophoretic profile of extracellular protein induced by cell walls of *Rhizoctonia solani*. *Journal of General Microbiology* **132**, 2345-2352.
- RODRIGUEZ-KABANA, R., GODOY, G., MORGAN-JONES, G. & SHELBY, R. A. (1983). The determination of soil chitinase activity: conditions for assay and ecological studies. *Plant and Soil* **75**, 95-106.
- SCHNEIDER, E. F., BARRAN, L. R., WOOD, P. J. & SIDDIQUI, I. R. (1977). Cell wall of *Fusarium sulphureum*. II. Chemical composition of the conidial and chlamyospore walls. *Canadian Journal of Microbiology* **23**, 763-769.
- SEDMAN, J. J. & GROSSBERG, S. E. (1977). A rapid, sensitive and versatile assay for protein using Coomassie brilliant blue G250. *Analytical Biochemistry* **79**, 544-552.
- SIVAN, A. & CHET, I. (1986). Biological control of *Fusarium* spp. in cotton, wheat and muskmelon by *Trichoderma harzianum*. *Journal of Phytopathology* **116**, 39-47.
- SIVAN, A., UCKO, O. & CHET, I. (1987). Biological control of *Fusarium* crown rot of tomato by *Trichoderma harzianum* under field conditions. *Plant Disease* **71**, 587-592.
- SIVAN, A. & CHET, I. (1989). The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. *Phytopathology* (in the Press).
- TOKIMOTO, K. (1982). Lysis of the mycelium of *Lentinus edodes* caused by mycolytic enzymes of *Trichoderma harzianum* when the two fungi were in antagonistic state. *Transactions of the Mycological Society of Japan* **23**, 13-20.