Effect of Trichoderma harzianum on Botrytis cinerea Pathogenicity

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ABSTRACT


Germination and germ-tube elongation of conidia of the pathogen Botrytis cinerea on bean leaves were reduced in the presence of the biocontrol agent Trichoderma harzianum T39. A reduction of 20 to 50% in germ-tube biomass was observed 24 h after inoculation. This reduction in germination did not result in complete prevention of disease development on the leaves. One day after inoculation, disease severity on leaves infected by the pathogen with and without the biocontrol agent was similar (>10% necrotic area). Subsequently, the disease developed rapidly in the control leaves and caused almost complete necrosis, whereas in the presence of T. harzianum T39 the necrotic area reached only ~50% of the leaf surface. The production of pectin-degrading enzymes by B. cinerea was measured up to 4 days after inoculation. Up to 1.3 enzyme units of polygalacturonase (PG), 9 microequivalents of NaOH, which express the activity of pectin methyl esterase (PME), and up to 1.5 units of pectate lyase (PL) were detected on bean leaves. Under the same conditions, the biocontrol agent, T. harzianum T39, did not produce any of these enzymes. On leaves infected with B. cinerea in the presence of the biocontrol agent, the activity of the pathogen’s PG was reduced by 40 to 83%. This was reflected on an activity gel by the faintness of these PG isoenzymes and the delay in their appearance. An up to 100% reduction in PME activity and a ~30% reduction in PL activity also were recorded. We suggest that T. harzianum T39 acts by reducing the enzyme activities of the pathogen. An indirect effect of enhancing the defense mechanisms of the host plant is discussed.

Additional keywords: cell wall-degrading enzymes, gray mold.

The fungus Botrytis cinerea Pers.:Fr. infects stems, leaves, flowers, and fruits of various crops (18). Trichoderma harzianum T39 is an efficient biocontrol agent that is used against this pathogen under commercial conditions (19,20,37,44). Several mechanisms have been suggested as being responsible for the control of plant diseases by Trichoderma spp. (6). Competition for space and nutrients is thought to control B. cinerea, because the pathogen requires exogenous nutrients for germination and germ-tube elongation over a period of several hours on the phylloplane before penetrating the host plant (4,13). Mycoparasitism against B. cinerea has been suggested by Bélanger et al. (2) and Tronson and Raa (39). Lorito et al. (31) reported antifungal activity against B. cinerea by chitinolytic enzymes of T. harzianum. Production of inhibitory compounds (7) or antibiotics (14,27), suppression of sporulation (25), and induced resistance (17) all may be involved in the control of this disease.

Extracellular pectolytic enzymes of fungal pathogens play an important role in pathogenesis (8,16). A variety of pectic enzymes are produced by B. cinerea, among them hydrolases such as polygalacturonase (PG) (42,23,28,33) and pectin methyl esterase (PME) (21) and lyases such as pectate lyase (PL) (35). Pectin-degrading enzymes disrupt the structure of plant cell walls (1). Fragments released from the cell wall by these enzymes can elicit plant defense responses (5,22). The effect of T. harzianum T39 on the activity of the pectin-degrading enzymes of B. cinerea and on disease development on bean leaves was studied to elucidate a new potential mechanism of biological control.

MATERIALS AND METHODS

Fungal isolates. The fungal pathogen, B. cinerea (isolate 16), was isolated from infected cucumber fruits in Israel (15) and maintained on potato dextrose agar (PDA) at 18 to 20°C. The biocontrol agent, T. harzianum T39, which was isolated from a cucumber canopy in a greenhouse, and the isolates T. viride T99 and T. harzianum T28, which were obtained from a collection (46), were maintained on PDA at 20 to 23°C.

Plant material. Bean plants (Phaseolus vulgaris L.) were planted in 2-liter polyethylene pots and grown in the greenhouse at 20 to 25°C. Leaves were collected from 3- to 4-week-old plants and incubated horizontally in polyethylene boxes on a polyethylene grid overlaid with water-soaked filter paper. The boxes were kept in transparent polyethylene bags to allow for condensation at vapor pressure deficit 0.104 to 0.118 kPa and 95% relative humidity. The boxes were held in a walk-in growth chamber at 18 to 20°C throughout the experiment (17).

Treatment of plant material. Detached leaves were inoculated with 50-μl drops containing suspensions of B. cinerea alone (5 × 10⁵ conidia per ml) or with T. harzianum T39 (10⁶ conidia per ml). To enhance disease development, the suspensions were supplemented with KH₂PO₄ as specified for each experiment. According to Van den Heuvel and Waterreus (41), phosphate stimulation of B. cinerea infection in French bean leaves is due to stimulated penetration via enhancement of the activities of certain pectic enzymes. Noninoculated leaves were treated with water. Each treatment contained five leaves as replicates; each replicate was inoculated with 25 drops. At each sampling (one a day for 5 days after inoculation), the severity of the symptoms under the drops was evaluated. Drops of each replicate were collected and centrifuged.

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to allow sedimentation of conidia. After centrifugation, pectin-degrading enzyme activity was monitored in the supernatant: activity gels were used to detect PG isoenzymes, and Western blot analysis was used to detect endo-PG.

**Estimation of the pathogen biomass.** Five drops of each treatment were observed under a light microscope (17) to estimate the *B. cinerea* biomass. Aniline blue was added to the drops 15 min before observation. Conidia (100 from each treatment, with or without *T. harzianum*) were evaluated for germination, and the length of the germ tubes was measured. The relative biomass was calculated by multiplying the percentage of germinating conidia by the average germ-tube length (17). Because variation in the latter parameter was small among replicates within treatments, this calculation was deduced a good estimation of the germ-tube biomass. After the first day of incubation the amount of hyphae on the leaf surface was estimated by light microscopy.

**Evaluation of disease severity.** Symptom severity on the drop-inoculated leaves was evaluated based on a 0 to 7 index, where 0 = symptomless leaf tissue; 1 = 1 to 12%, 2 = 13 to 25%, 3 = 26 to 50%, and 4 = 51 to 100% necrotic area under the drop; and 5 = expansion zone around the drop <2 mm, 6 = expansion zone 2 to 5 mm, and 7 = expansion zone >5 mm (12).

**Statistical analysis.** Experiments were arranged in a completely randomized design and repeated at least three times. Treatments were replicated at least four times. Data were arcsine transformed, subjected to analysis of variance, and tested for significance by the Student-Newman-Keuls multiple range test. Only the findings of one representative experiment are presented below.

**Enzymatic activity assays and protein determinations.** PG (EC 3.2.1.15) activity was tested by a modified Nelson-Somogyi assay (23). The reaction mixture contained 2% (wt/vol) polygalacturonic acid (Sigma Chemical Company, St. Louis) in 50 mM acetate buffer (pH 5.2) as the substrate. One unit was defined as the amount of enzyme that released 1 μM galacturonic acid in 1 h. PME (EC 3.1.1.11) activity was determined by a modification of the continuous titration method. A solution of 1.2% (wt/vol) pectin (Sigma), adjusted to pH 5.5 with 1 M NaOH, served as the substrate. After the addition of 1 ml of the crude enzyme to 3 ml of the substrate, the exact pH was measured (Spectronic 401, Spectronic Instruments, Rochester, NY). After 1 h of reaction at 30°C, the enzyme-substrate mixture was titrated with 0.02 N NaOH to the pH recorded at zero time. Data are expressed as micromoles of NaOH absorbed per hour per milliliter of crude enzyme (21). PL (EC 4.2.2.2) activity was determined spectrophotometrically at 235 nm. The reaction was a modification of the assay suggested by Nasuno and Starr (36). The reaction mixture contained 0.3% (wt/vol) polygalacturonic acid in 50 mM Tris-HCl buffer (pH 8.5) in the presence of 1 mM CaCl2 as the substrate. One unit of activity was defined as the amount of enzyme that caused an increase of 2.6 in absorbance at 235 nm, equivalent to the release of 1 μmol aldehyde groups (36). Protein concentration was measured spectrophotometrically at 595 nm with Bradford reagent (Bio-Rad Laboratories, Munich).

**Gel electrophoresis.** Polyacrylamide gel electrophoresis was performed by PhastSystem (Pharmacia LKB Biotechnology, Uppsala, Sweden) with 7.5% native gels according to the manufacturer’s directions. The drops collected from inoculated leaves were concentrated by Cintercon-10 (Amicon Co., Beverly, MA). Proteins were quantified, and samples were brought to the same protein concentration, i.e., 0.1 to 0.5 μg. Sucrose (10%, wt/vol) was added to the analyzed samples. Identification of PG on polyacrylamide gels was carried out as reported by Lisker and Retig (30). For

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**Fig. 1.** The effect of KH2PO4 concentration on the biomass of *Botrytis cinerea*, with (●) or without (○) *Trichoderma harzianum* T39, on bean leaves 20 h after inoculation. Germ-tube biomass = percent germination × germ-tube length. Vertical bars indicate standard error.

**Fig. 2.** A. Effect of *Trichoderma harzianum* T39 on *Botrytis cinerea* supplemented with various KH2PO4 concentrations 2 days after inoculation on bean leaves. A: Effect of polygalacturonase (PG) activity. One unit = the amount of enzyme that released 1 μM galacturonic acid in 1 h. B. *cinerea* alone (●); *B. cinerea* and *T. harzianum* T39 (○). Vertical bars indicate standard error. B. Effect of *T. harzianum* and KH2PO4 concentrations on disease severity. Disease severity was indexed on a scale of 0 to 7, where 0 = healthy leaf and 7 = expansion zone of necrotic lesions >5 mm. *B. cinerea* alone (●); *B. cinerea* and *T. harzianum* T39 (○). Vertical bars indicate standard error.
staining, gels were incubated for 30 min with 1.2% (wt/vol) poly-
galacturonase and immersed for an additional 15 min in 0.05%  
(wt/vol) ruthenium red (Sigma) solution. Destaining in water en-
hanced the appearance of the bands.

**Western blot analysis.** Samples containing 10 μg of protein were  
separated by 10% sodium dodecyl sulfate-polyacrylamide gel  
electrophoresis (SDS-PAGE) (26) by the Mini Protein II dual slab  
cell system (Bio-Rad, Hercules, CA). Electrophoresis was carried  
out at 150 to 200 V for 1 h. Proteins were electrophoretically transferred to a nit-
trocellulose membrane with a Bio-Rad mini transfer cell for 45 min.  
The membrane was blocked with 3% (wt/vol) skim milk in Tris-
HCl-buffered saline (TBS), pH 7.6, for 2 h at room temperature.  
The membrane was probed in TBS for 2 h with specific sera (provided  
by B. Williamson, Scottish Crop Research Institute, Inver-
gowrie, Scotland). The membrane was washed three times, 10 min  
each, in TBS containing 0.5% (vol/vol) Tween 20 (TBST). The  
membrane was incubated for 2 h with alkaline phosphatase-labeled  
goat anti-rabbit antibody (Sigma) in TBST. After three 10-min washes  
in TBST, color was developed in Tris-NaCl buffer, pH 9.8,  
containing 5 mM MgCl₂-6H₂O, utilizing 5-bromo-4-chloro-3-indolyl  
phosphate/nitroblue tetrazolium alkaline-phosphatase substrates  
(Boehringer GmbH, Mannheim, Germany) (24). The size of the en-
zyme was estimated by comparison to prestained SDS-PAGE, stand-
ard low range molecular weight markers (Bio-Rad, Hercules, CA).

**RESULTS**

**Effect of T. harzianum on B. cinerea germination and biomass on bean leaves.** In the presence of T. harzianum T39, B.  
cinerea biomass was reduced by 20% to 50% at all KH₂PO₄ concen-
trations tested (Fig. 1). This reduction was due mainly to an  
effect on germ-tube elongation; the germination rate was only par-
tially affected. Although T. harzianum T39 reduced the biomass of  
the pathogen after 20 h, many conidial germ tubes invaded the  
host tissue, and disease symptoms appeared. Symptom severity on  
leaves treated with B. cinerea with or without T. harzianum was  
similar after 24 h (up to 12% necrotic area). However expansion  
of the lesions was slowed (by as much as 50%) in the presence of  
T39. Therefore, we speculated that T. harzianum T39 may affect  
the activity of the pectin-degrading enzymes of B. cinerea.

**Effect of KH₂PO₄ on PG activity and disease severity of B.  
cinerea on bean leaves.** B. cinerea exhibited high PG activity in  
vitro (G. Zimand, A. Kapat, Y. Elad, and I. Chet, unpublished data)  
and on bean leaves (Fig. 2A). The highest level of B. cinerea PG  
activity was observed in the presence of 4 g of KH₂PO₄ per liter  
after 2 days of incubation (Fig. 2A). T. harzianum also exhibited  
high PG activity in vitro (G. Zimand, A. Kapat, Y. Elad, and I.  
Chet, unpublished data) but not on bean leaves. In the presence of  
T. harzianum, the activity of B. cinerea PG was reduced. The  
amount of this reduction was affected by the KH₂PO₄ concen-
tration, with PG activity more pronounced at the highest concen-
tration. The reductions in enzyme activity at 0.5, 1, and 4 g of  
KH₂PO₄ per liter were 66, 66, and 81%, respectively. Disease  
severity on control leaves inoculated only with B. cinerea reached  
a symptom-severity index value of 3.4, whereas leaves treated with  
T. harzianum T39 only reached a value of 1 after 2 days of incub- 
ation (Fig. 2B). The greatest reductions in disease severity as  
well as in PG activity (81%) were achieved at the highest concen-
tration of KH₂PO₄ (4 g/liter) tested.

**Disease development on bean leaves as affected by the bio-
control agent and 4 g of KH₂PO₄ per liter.** Disease severity was  
low (index value of 1 to 2) in treatments with or without T. har-
zianum T39 after 1 day of incubation. Disease severity increased  
to an index value of 4.5 and 7 in the control treatment and to 1.6  
and 3.2 in the presence of T. harzianum T39 after 2 and 4 days,  
respectively. The differences between the treatments with and with-
out T. harzianum were not as pronounced after 2 to 4 days relative  
to the first day of incubation (Fig. 3).

**Electrophoresis of proteins collected from the surface of in-
fected bean leaves.** PG activity in crude protein samples collected  
from bean leaves infected by B. cinerea in the presence or absence  
of T. harzianum T39 was analyzed on native polyacrylamide gels  
(Fig. 4). After 1 day of incubation in the absence of T. harzianum  
T39, B. cinerea had produced three distinct PG isoenzymes. In  
contrast, isoenzyme activity was weak in the samples taken from  
leaves infected with B. cinerea in the presence of T. harzianum.  
After 2 days on bean leaves, B. cinerea had produced six isoen-
zymes, whereas in the presence of T. harzianum only three of  
these were evident, the same number that was observed on the  
first day in the B. cinerea treatment. After 5 days on bean leaves,  
the six isoenzymes observed on the second day were most pro-
nounced. In the presence of T39, six weak isoenzymes were ob-
erved (Fig. 4).

**Western blot analysis with specific endo-PG antibodies of the  
crude proteins collected from the leaves.** Western blot analysis of  
drops collected from the treated leaves revealed one band with  

![Fig. 3. Effect of Trichoderma harzianum T39 on severity of disease caused by Botrytis cinerea 1 to 4 days after inoculation: B. cinerea alone (●) or with T. harzianum T39 (■). Disease severity was indexed on a scale of 0 to 7, where 0 = healthy leaf and 7 = expansion zone of necrotic lesions >5 mm. Vertical bars indicate standard error.](image)

![Fig. 4. Electrophoretic patterns on native gel of polygalacturonase produced by Botrytis cinerea on bean leaves with and without Trichoderma harzianum T39 1, 2, and 5 days after inoculation. Lanes 1, 3, and 5, B. cinerea alone; lanes 2, 4, and 6, B. cinerea and T. harzianum T39.](image)
TABLE 1. Effect of *Trichoderma* isolates on polygalacturonase (PG) activity and disease severity of *Botrytis cinerea*

<table>
<thead>
<tr>
<th>Incubation time (days)</th>
<th>Disease severity</th>
<th>PG (U)</th>
<th>Disease severity</th>
<th>PG (U)</th>
<th>Disease severity</th>
<th>PG (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.47 a</td>
<td>0.18</td>
<td>3.18 a</td>
<td>1.20</td>
<td>6.2 a</td>
<td>1.00</td>
</tr>
<tr>
<td>T39</td>
<td>0.46 b</td>
<td>0.03</td>
<td>1.58 b</td>
<td>1.34</td>
<td>2.5 b</td>
<td>1.10</td>
</tr>
<tr>
<td>T28</td>
<td>1.85 a</td>
<td>0.90</td>
<td>3.65 a</td>
<td>1.34</td>
<td>6.4 a</td>
<td>1.30</td>
</tr>
<tr>
<td>T99</td>
<td>1.62 a</td>
<td>0.15</td>
<td>2.70 a</td>
<td>1.17</td>
<td>5.5 a</td>
<td>1.28</td>
</tr>
</tbody>
</table>

a Disease severity index on a scale of 0 to 7, where 0 = healthy leaf and 7 = expansion zone of necrotic lesions >5 mm.

b One unit = the amount of enzyme that released 1 µM galacturonic acid in 1 h.

c Numbers within columns followed by the same letter do not differ significantly at P ≤ 0.05. The Student-Newman-Keuls multiple range test was used for analysis. No variability in enzymatic activity was detected in any of the treatments under these conditions.

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**Fig. 5.** Western blot analysis (10% gel) of *Botrytis cinerea* polygalacturonase (PG) with and without *Trichoderma harzianum* T39 on bean leaves. The enzyme samples were: lanes 1, 3, and 5, *B. cinerea* alone; lanes 2, 4, and 6, *B. cinerea* with *T. harzianum* T39, supplemented with 4 g of KH₂PO₄ per liter; lane 8, *B. cinerea* alone; and lane 9, *B. cinerea* with *T. harzianum* T39, supplemented with 0.5 g of KH₂PO₄ per liter. Pairs of samples for lanes 1 and 2, 3 and 4, and 5 and 6 were taken 1, 2, and 5 days after inoculation, respectively. Samples for lanes 8 and 9 were taken 4 days after inoculation. A molecular mass of ~36 kDa in control leaves infected with *B. cinerea* alone, supplemented with 4 g of KH₂PO₄ per liter (Fig. 5). This band was similar to the endo-PG band described by Johnston and Williamson (23). It appeared weakly after 1 day of incubation in the control treatment (lane 1) and did not appear when *T. harzianum* was present on the leaves (lane 2). After 2 days of incubation, this band was stronger in the control treatment (lane 3) and was faintly apparent in the treatment with *T. harzianum* (lane 4). A similar phenomenon was observed after 5 days of incubation (lanes 5 and 6). The results were similar when the leaves were inoculated with 0.5 g of KH₂PO₄ per liter (lanes 8 and 9) (Fig. 5).

**Effect of other *Trichoderma* spp. isolates on *B. cinerea* disease development and PG activity.** The effects of *Trichoderma* isolates T99 and T28 were compared to that of the biocontrol agent, T39 (Table 1). Only T39 significantly reduced disease severity and PG activity of the pathogen after 1 and 2 days of incubation. Although PG activity was not reduced by T39 on the third day of incubation, disease severity was affected by the previous reductions in enzyme activity (Table 1).

**Effect of *T. harzianum* on *B. cinerea* PME and PL activities on bean leaves.** *T. harzianum* T39 did not produce any PME or PL on bean leaves. The activities of PME and PL produced by *B. cinerea* on bean leaves supplemented with 4 g of KH₂PO₄ per liter and *T. harzianum* were reduced by approximately 30 and 100%, respectively, relative to the corresponding activities in the absence of the biocontrol agent (Fig. 6).

**DISCUSSION**

Disease severity was similar on bean leaves inoculated with *B. cinerea* with or without *T. harzianum* T39, as determined 20 h after inoculation. At the same time, the biomass of the pathogen was reduced in the presence of *T. harzianum* T39, mainly due to an effect on germ-tube elongation and, to a lesser extent, on germination rate. In the presence of *T. harzianum* T39, germination of *B. cinerea* conidia and subsequent penetration of host tissue occurred, as was evident based on the initial formation of symptoms. However, although disease severity on the control leaves developed rapidly, it progressed much more slowly on the leaves treated with the biocontrol agent.

Because pectic enzymes are considered the principal enzymes involved in pathogenicity (41), we studied the effect of the biocontrol agent on the pectin-degrading enzymes of the pathogen on leaves. Borowicz et al. (3) previously suggested inhibition of fungal enzymes by the plant growth-promoting bacteria *Pseudomonas* spp. in vitro.

Very little information is available concerning the activity of the pectic enzymes of *T. harzianum* in vivo. In our study, no PG, PME, or PL activity was detected on leaves treated with *T. harzianum* T39 alone. These results are in contrast to findings under in vitro conditions (G. Zimand, A. Kapat, Y. Elad, and I. Chet, unpublished data) and demonstrate the fact that fungi can produce different sets of enzymes as a function of growth conditions, as suggested by Hancock et al. (21).

Activities of PG, PME, and PL in *B. cinerea*, as well as disease severity, were high on bean leaves (Figs. 2A and 6), similar to severity on other hosts, such as carrot, strawberry, raspberry, cabbage, and grapes (35). Two to four days after inoculation, while the amount of hyphae on leaves inoculated with *B. cinerea* alone and in the presence of *T. harzianum* T39 seemed to be the same, the activity of the pectin-degrading enzymes and disease severity were reduced in the presence of the biocontrol agent (Figs. 2A and 6). These reductions could be due simply to the reduction in *B. cinerea* biomass during the initial 20 h after inoculation, but they also could be a direct or indirect effect of *T. harzianum* T39 on the *B. cinerea* enzymes. Our previous results (45) indicated that *T. harzianum* T39 inoculated on bean leaves did not produce any detectable substances inhibitory to several microorganisms, including *B. cinerea*. For this reason, direct and indirect effects of *T. harzianum* T39 on the *B. cinerea* enzymes were considered here. *T. harzianum* T39 could manifest a direct effect on enzyme production, activation, or secretion of enzymes. Such an effect has been suggested by Milling and Richardson (34) and Daniels and Lucas (10) as the mode of action for the fungicide pyrimethanil against *Botrytis*. Alternatively the direct effect could be similar to that suggested by Piet et al. (38), i.e., secretion of proteolytic enzymes that affect the pathogen’s enzymes. Indirect effects of *T. harzianum* T39 could involve a leaf’s defense mechanisms.

Many authors have reported that PG enzyme group activity is a major component of the infection process (1,9) and that they are the first enzymes secreted by germinating and nongerminating conidia of *B. cinerea* (29,43). For this reason, PG activity was selected for further study. *B. cinerea* produces a number of isoenzymes in vitro and in vivo belonging to the PG group (11,29,41). The production of several isoenzymes of PG has been reported for other pathogens, including *Sclerotinia sclerotiorum* (32).
The effect of *Trichoderma harzianum* T39 on the activity of the pathogen's enzymes was tested in the presence of various concentrations of KH₂PO₄. Van den Heuvel and Waterreus (41) suggested that KH₂PO₄ enhances disease development and PG activity. Therefore, we tested possible competition between the pathogen and biocontrol agent for this compound, which may cause a reduction in pectic enzyme activity. However, we found no evidence of such competition on bean leaves.

In the presence of *T. harzianum* T39 on bean leaves, the production of some PG isoenzymes of the pathogen are delayed (Figs. 4 and 5), and their activities are reduced (Fig. 4). We suggest that the effect on disease severity is a combination of two phenomena: the first is a direct effect of *T. harzianum* T39 on the enzyme's activities. However, because the direct effect on enzyme activity may not be sufficient to cause such disease reduction, a possible combination with an indirect effect on a leaf's defense mechanism is suggested. Urbanek et al. (40) found that *B. cinerea* PG elicits defense mechanisms in bean leaves. Cervone et al. (5) suggested that the accumulation of pectic enzyme products, i.e., oligogalacturonides with a degree of polymerization higher than nine, act as elicitors of defense mechanisms of plants. We suggest that in the presence of *T. harzianum* T39, the intensity of the pectolytic enzyme activity is reduced. Therefore, the bigger oligogalacturonides may accumulate and elicit the host plant's defense mechanisms, slowing the development of disease severity. Indeed, under conditions in which no control was achieved by the biocontrol agent, no reduction in PG activity was found. Moreover, other *Trichoderma* isolates did not reduce disease severity and did not affect PG activity (Table 1).

**LITERATURE CITED**


18. Elad, Y., and Shitonenberg, D. 1995. *Botrytis cinerea* in greenhouse vegetables: Chemical, cultural, physiological and biological controls and their...