Permeability Changes in Hyphae of *Rhizoctonia solani* Induced by Germling Preparations of *Trichoderma* and *Gliocladium*

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ABSTRACT

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Water extracts of germlings (young, actively growing hyphae on bran) of isolates of *Trichoderma* spp. and *Gliocladium virens* affected growth of *Rhizoctonia solani* (R-23) in liquid culture. Bran control and germling extracts from 7 of 14 isolates increased mycelium weight up to fourfold. Extracts from one *T. viride* isolate (TS-1-R3), one *T. hamatum* isolate (31-3), and all four *G. virens* isolates (Gl-3, Gl-9, Gl-17, Gl-21) prevented growth as effectively as 0.2 mM $HgCl_2$. Similar results were obtained with three other isolates of *R. solani* investigated. Leakage of compounds from mycelial mats of R-23 was induced after mats were exposed to germling extracts of *T. harzianum* (Th-5), *T. hamatum* (Tm-23, 31-3), or *G. virens* (Gl-3, Gl-9, Gl-17, Gl-21) for less than 0.5 hr. The chemical composition of the leaked materials (soluble protein, carbohydrate, amino acids, salts) was determined by colorimetry and conductance. Leakage was accompanied by a reduction in the mycelial weight of R-23. The rate of leakage indicated an immediate and gradually increasing loss of materials from pathogen hyphae. Extracts from young germling preparations induced more leakage than extracts from older bran preparations or conidia on bran. Heat treatment of extracts (121 C) reduced leakage by 75–80%. Addition of Gl-21 germlings to soil containing noninfested beet seed or seed infested with R-23 resulted in production of membrane-altering substances in the seed extracts that induced leakage from mycelial mats of R-23.

In the biological control of soilborne plant pathogens by other microorganisms, destruction of the pathogen by mycoparasitism or antibiosis is a major mechanism for disease control (17,18). As a prerequisite for cell death, it is apparent that the cell wall or membrane must be irreversibly disrupted.

When plant pathogens attack host cells, the enzymes or phytotoxins produced by the pathogen adversely affect the host tissue cell wall and/or membrane (5,10,21,24). Some toxins cause rapid and drastic changes in the permeability of susceptible plant tissue membranes; as a consequence, the water balance is altered, cells become leaky and lose electrolytes and other materials, uptake of nutrients and accumulation of minerals are inhibited, and electrochemical potential drops. Similar phenomena are observed when enzymes, antibiotics, and other chemicals, either naturally formed or synthesized, effectively destroy membrane permeability in plant pathogens (3,4,8,10,11,16). There is often a decrease in mycelial weight, increased protein leakage, reduced glucose uptake, and morphological changes in the hyphae accompanied by protoplasmic bursting.

Several peptide antibiotics such as alamethicin, paracelsin, or trichotoxin produced by *Trichoderma* and *Gliocladium* isolates, which are noted for their biocontrol capabilities (17), also altered membrane permeability (1,2,7). However, research with these metabolites has mostly been concerned with their effects on synthetic membranes or erythrocytes rather than on membranes of plant pathogens. Such metabolites were shown to be hemolytic and also to alter the ionic flow across synthetic bilayer lipid membranes (1,6,9).

Metabolites from culture filtrates of *Trichoderma* or *Gliocladium* isolates were reported in the early work of Weindling (23) to adversely affect plant pathogen membranes. In 1934, he suggested that a diffusible "toxic principle" caused considerable vacuolation and coagulation of *Rhizoctonia* and *Pythium* hyphae so that the cells appeared empty with only slight wall damage. Although reports indicate pathogen cell wall disintegration by isolates of *Trichoderma* and *Gliocladium* (4), very little definitive information has appeared on pathogen membrane disruption by these isolates. This report describes the leakage of cell constituents from hyphae of the pathogen *R. solani* Kühn after membrane damage by germling extracts of isolates of *Trichoderma* spp. and *G. virens* Miller, Giddens & Foster and the production of membrane-altering metabolites in a natural soil.

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MATERIALS AND METHODS

Fungus isolates and preparations. The ability of extracts from biocontrol preparations to induce cytoplasmic leakage of *R. solani* was generally studied using isolate R-23, obtained from diseased cotton plants grown in Mississippi. Other isolates studied were R-103 and R-18 from diseased tomato plants in Maryland and South Carolina, respectively, and R-108 from potato tubers in Maryland. Cultures were maintained on potato-dextrose agar and grown as indicated in each experiment.

Ten isolates of *Trichoderma* spp. and four isolates of *G. virens* from the Soilborne Diseases Laboratory collection were used (12,13). These included three isolates of *T. viride* Pers. ex Gray (T-1-R4, T-1-R9, TS-1-R3), four of *T. harzianum* Rifai (WT-6-24, Th-5, Th-58, Th-23-R9), three of *T. hamatum* (Bonord.) Bain. (TRI-4, Tm-23, 31-3), and four of *G. virens* (Gl-3, Gl-9, Gl-17, Gl-21). Cultures were maintained on V-8 juice agar (200 ml of V-8 juice, 800 ml of water, 1 g of glucose, 20 g of agar, and 6.0 ml of 1.0 N NaOH) at 23–26 C. Conidial suspensions were prepared from 9-day-old colonies growing on this medium. Wheat bran (100 g) was passed through a sieve with 1-mm openings, mixed with water (100 ml), and autoclaved in 1-L Erlenmeyer flasks for 1 hr. After the medium had cooled, 10 ml of a spore suspension, counted with a hemacytometer, was added to each flask to provide 10^7 conidia per 100 g of bran. The cultures were incubated 0, 3, 10, 15, and 40 days at 25 C. At 0 days of incubation, the preparation consisted of conidia on bran. Three-day-old preparations, hereafter referred to as germlings, contained young, actively growing hyphae on bran with little evidence of resting spores (12,13). With increasing incubation time, the preparations contained less active mycelium with greater amounts of conidia and chlamydospores formed as a result of fungal growth and sporulation on the bran.

Extract preparations. After the appropriate incubation times, bran cultures in flasks were mixed with 200 ml of distilled water and shaken gently on a wrist-action shaker for 1 hr. Flask contents were poured over a triple layer of cheesecloth and the collected extract was centrifuged at 2,000 *g* at 5 C for 25 min. For studies on leakage, the clarified supernatant was used without further processing. When the extract was used in growth studies, it was recentrifuged at 5,000 *g* for an additional 30 min and filter-sterilized through a Metricel membrane filter (0.45 μm).

Effect of extracts on growth of *R. solani* and cytoplasmic leakage. Ten-milliliter portions of 1.0% potato-dextrose broth were placed in 100-ml Erlenmeyer flasks and the flasks were autoclaved for 20 min. After the flasks cooled, 20 ml of the sterile extracts of each of the 10 *Trichoderma* and 4 *G. virens* isolates was added aseptically to each flask. Controls contained 20 ml of sterile water, a sterile solution of $HgCl_2$ (0.2 mM), or sterile bran extract. An agar plug (5 mm) from the edge of a 3-day-old potato-dextrose agar culture of *R. solani* (R-23) was placed in each flask and the flasks were incubated in the dark at 23–25 C. After 5 days of static incubation, the mycelial mats were harvested, dried at 80 C, and weighed. Germling extracts of selected isolates of *Trichoderma* (TS-1-R3, 31-3) and *G. virens* (Gl-3, Gl-21) were used in an additional study to determine extract effects on growth of several other *R. solani* isolates (R-18, R-103, R-108).

Several methods were adapted to determine the effect of extracts on leakage of cell constituents from *R. solani* (5,7,21). Forty-milliliter portions of extract and appropriate controls were placed in glass jars (8.5 × 8.5 cm) with 10 ml of an osmoticum that provided 0.1 M mannitol and 1.0 mM $CaSO_4$ (5). Since the pH of extracts was in the range of 6.2–6.5, control solutions were adjusted to pH 6.3 with a dilute NaOH solution. In one experiment, extracts from 10-, 15-, and 40-day-old bran preparations of *T. hamatum* (31-3) and *G. virens* (Gl-21) were used in addition to germling extracts. Four 6-day-old mycelial mats of *R. solani* (R-23) grown on 10 ml of 0.5% potato-dextrose broth in 60-mm-diameter petri plates were washed with tap water three times and placed in each jar. The jars were shaken on an orbital shaker at about 125 rpm for 3 hr, except for one experiment in which they were shaken for 0.5, 1, 2, 3, and 4 hr. After these times, mycelial mats were removed gently from the extract with a rubber policeman, washed in tap water four times, and placed in jars measuring 8.5 × 8.5 cm containing 50 ml of distilled water. After an additional 3 hr of shaking, mycelia were separated from the liquid by filtration through filter paper, the papers with mycelia were dried at 80 C, and dry weight of the mycelial mats was determined. In one experiment, mycelia were recovered after 0.5, 1, 2, and 3 hr in the water. The effect of heat treatment on activity of the extracts was determined by placing mycelial mats of *R. solani* (R-23) in extracts that were autoclaved for 15 min and cooled.

TABLE 1. Effect of germling extracts of isolates of *Trichoderma* spp. and *Gliocladium virens* on growth of several isolates of *Rhizoctonia solani* in liquid medium[y]

Isolate	Mycelial dry wt of *R. solani* isolates (mg/30 ml)			
	R-23	R-18	R-103	R-108
Control (water)	56 d[z]	70 b	50 b	47 b
$HgCl_2$ (0.2 mM)	1 e	3 d	2 d	5 d
Bran	204 ab	180 a	153 a	157 a
T. viride				
T-1-R4	104 c			
T-1-R9	117 c			
TS-1-R3	7 e	20 c	24 c	19 cd
T. harzianum				
WT-6-24	214 a			
Th-5	104 c			
Th-58	200 ab			
Th-23-R9	185 b			
T. hamatum				
TRI-4	101 c			
Tm-23	65 d			
31-3	9 e	19 c	8 d	23 c
G. virens				
Gl-3	12 e	11 cd	5 d	9 cd
Gl-9	6 e			
Gl-17	11 e			
Gl-21	10 e	4 d	6 d	5 d

[y] Culture liquid contained 10 ml of 1.0% potato-dextrose broth and 20 ml of germling extract, water, or $HgCl_2$. Dry weight determined after 5 days of incubation.

[z] Numbers in each column followed by same letter do not differ significantly from each other at $P = 0.05$ according to Duncan's multiple range test.

Assay for leakage materials. After removal of the mycelial mats, the solution filtrates were assayed for various materials without further purification. Soluble carbohydrate was determined with anthrone reagent using glucose as the standard (15). Ninhydrin reagent was used to quantify compounds containing amino groups, mainly amino acids, but also imino acids, amino alcohols, amides, and the ammonium ion (20). Glycine was the standard for the assay. The amount of glucose was determined with the glucose oxidase reagent (Sigma Chemical Co., St. Louis, MO). Soluble protein was assayed with the Folin phenol reagent using egg albumen as the standard (14). The method of Reissing et al (19) was used to determine acetylglucosamine. Values for all constituents were expressed as milligrams per 100 mg dry weight of *R. solani* mycelium. Electrolyte, or soluble salt, leakage was measured as conductance (μmhos/100 mg of mycelium) with a conductivity bridge.

Production of a membrane-altering material in natural soil. Beet seed, noninfested or infested with *R. solani*, was added to a natural soil together with a mycelial preparation of Gl-21 to determine whether a membrane-altering material was produced by a biocontrol fungus under natural conditions and in response to a pathogen. Since *R. solani* is usually found in soil fractions as mycelium embedded in organic debris, pathogen-infested beet seed was used as inoculum to simulate this situation. Beet (*Beta vulgaris* L.) seed was mixed with water (1:1, w/v), placed in flasks, autoclaved, and seeded with an agar plug of *R. solani*. Preparations were incubated in the laboratory at 23–26 C for 28 days and then air-dried (12,13). This inoculum was used within 1 wk of drying, but it could be kept under normal laboratory conditions for several months without appreciable loss in viability.

Noninfested or infested beet seed (50 g) and germlings of Gl-21 (40 g) were mixed with 4 kg of air-dried loamy sand in polypropylene pans (12.5 × 23 × 43 cm) and the soils were moistened to −0.3 bar (8–9% moisture) as determined with a pressure plate extractor. The pans were covered with aluminum foil and incubated for 5 days at 22–25 C. Five days is sufficient for the *Gliocladium* isolate to invade beet seed, proliferate, and reduce survival of *R. solani* if the pathogen is present in the seed (12,13). Beet seed was retrieved, washed in running tap water, and blended for 3 min in 150 ml of water in an ice bath with a Sorvall Omnimixer at about 5,000 rpm. The suspensions were centrifuged the same way as the bran preparations described above. Mycelial mats of *R. solani* were placed in the supernatant with osmoticum for 3 hr, washed, and resuspended in distilled water for an additional 3 hr, after which the water solutions were analyzed for leakage constituents.

Statistical analyses. Each experiment was performed twice with four replications. All data were analyzed for significant differences using Duncan's multiple range test with the Statistical Analysis System computer program.

RESULTS

Effect of germling extracts on growth of *R. solani*. Extracts from several isolates of the three *Trichoderma* species (T-1-R4, T-1-R9, WT-6-24, Th-58, Th-23-R9, Th-5, TRI-4) as well as from the bran control increased growth of R-23 (Table 1). In contrast, extracts from all isolates of *G. virens*, one of *T. viride* (TS-1-R3), and one of *T. hamatum* (31-3) prevented growth of R-23 as effectively as $HgCl_2$. Similarly, germling extracts of isolates TS-1-R3, 31-3, Gl-3, and Gl-21 also reduced growth of other *R. solani* isolates (R-18, R-103, R-108). In some instances, extracts from Gl-3 and Gl-21 prevented growth more than those from TS-1-R-3 and 31-3, and

inhibition with Gl-3 and Gl-21 extracts was similar to that observed with $HgCl_2$.

Effect of extracts on leakage. Substances in several germling extracts induced significant leakage of chemical constituents from hyphae of R-23 (Table 2). Soluble proteins, amino acids, carbohydrates, and salts were leaked from hyphae into water after mycelial mats of R-23 were in contact with extracts of isolates of *G. virens* (Gl-3, Gl-9, Gl-17, Gl-21), *T. hamatum* (Tm-23, 31-3), and *T. harzianum* (Th-5). Extracts from 10 of the 14 isolates also reduced the weight of mycelial mats of R-23. The greatest leakage of constituents and reduction of mycelial weight occurred after contact of pathogen hyphae with germling extracts of the isolates of *G. virens*. In all cases, about 20% of the leaked carbohydrate was glucose. Acetylglucosamine was not detected in the water solutions. Extracts from 10 of the 14 isolates tested caused leakage of salts. $HgCl_2$, which is reported to disrupt membranes, generally did not induce leakage even though it slightly reduced mycelial weights.

The relationship between length of exposure of mycelial mats of R-23 to germling extracts of Gl-21 and amount of leakage of anthrone-positive materials was generally linear (Fig. 1). Most leakage occurred after mycelial mats of R-23 were exposed to germling extracts for 3–4 hr, although there was significant leakage after 0.5 hr. Dry weight of mycelium was reduced 22 and 44% during leakage after 0.5 and 4 hr of exposure to extracts, respectively. The rate of leakage from hyphae into water after mats were in contact with extracts for 3 hr was also determined. The leakage rate was highest during the first 2 hr after the mycelial mats were placed in the water: 44, 60, and 85% of the total leaked carbohydrate was present 0.5, 1, and 2 hr, respectively, after leakage began. Although the maximum amount of leakage materials over time was not determined, the rate curve began to level off after 2 hr of leakage.

The age of fungus-bran preparations of Gl-21 and 31-3 from which extracts were prepared influenced the effect of extracts on leakage. The greatest amount of leaked constituents, demonstrated by the presence of soluble carbohydrates, resulted from the activity of extracts from young (3- and 10-day-old) preparations. Extracts from old (40-day-old) preparations or conidia on bran (0-day-old) were ineffective, and extracts from 15-day-old preparations were intermediate. The soluble carbohydrates leaked from hyphae of R-23 in milligrams per 100 mg of mycelium after 3 hr of contact with extracts of 0-, 3-, 10-, 15-, and 40-day-old preparations of Gl-21 were 3.6c, 34.4a, 24.3ab, 16.7b, and 8.4c, respectively. Values followed by the same letter do not significantly differ from each other according to Duncan's multiple range test ($P = 0.05$). A similar trend, but with lower amounts, was observed with preparations of 31-3.

Heat treatment of extracts before their contact with mycelial mats of R-23 considerably reduced their abilities to induce leakage. Autoclaved extracts of Gl-21 and 31-3 germlings were 75–80% less effective than natural extracts in causing leakage of soluble carbohydrates or reducing mycelial weights of R-23.

Production of membrane-altering materials in soil. When Gl-21 germlings were added to soil containing beet seed, either noninfested or infested with R-23, membrane-altering materials

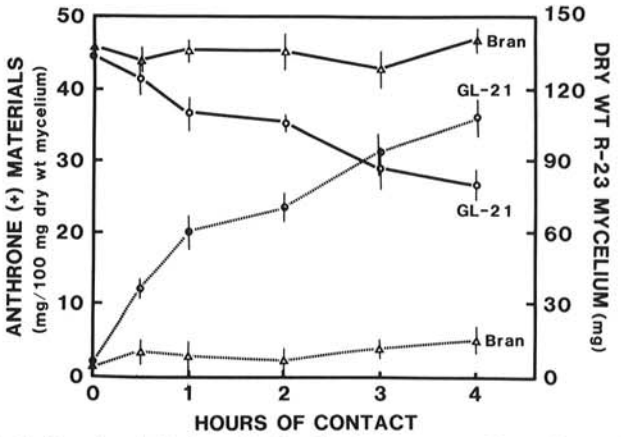

Fig. 1. Cytoplasmic leakage (broken lines), as measured by anthrone (+) materials, from hyphae of *Rhizoctonia solani* (R-23) after various hours of contact with germling extracts of *Gliocladium virens* (Gl-21) or bran extracts. After contact, mycelial mats were placed in distilled water for leakage for 3 hr and then dried for weight determination (solid lines). Bars indicate standard deviations.

TABLE 2. Cytoplasmic leakage from hyphae of *Rhizoctonia solani* (R-23) induced by germling extracts of isolates of *Trichoderma* spp. and *Gliocladium virens*[w]

Isolate	Leaked materials (mg/100 mg dry wt of mycelium)[x]				Residual dry wt of R-23 mycelium (mg)[y]
	Soluble protein	Anthrone (+)	Ninhydrin (+)	Electrolytes (μmhos)	
Control (water)	0.31 f[z]	0 f	0 e	13 e	143 ab
$HgCl_2$ (0.2 mM)	0.40 ef	1.22 ef	0.12 e	30 cd	116 cd
Bran	0.42 ef	2.20 ef	0 e	13 e	148 a
T. viride					
T-1-R4	0.62 def	1.30 ef	0 e	24 de	142 ab
T-1-R9	0.53 def	0.94 ef	0 e	20 de	144 ab
TS-1-R3	0.80 def	3.41 de	0.11 e	19 de	112 cde
T. harzianum					
WT-6-24	0.90 de	3.02 def	0.13 e	30 cd	128 bc
Th-5	1.12 d	5.95 d	0.40 d	37 c	94 def
Th-58	0.51 def	0.60 ef	0 e	40 c	134 abc
Th-23-R9	0.49 def	0.91 ef	0.11 e	28 cd	145 ab
T. hamatum					
TR1-4	0.80 def	1.15 ef	0 e	74 b	112 cde
Tm-23	2.92 b	18.16 b	0.94 b	82 b	87 f
31-3	1.63 c	10.94 c	0.43 cd	105 a	117 cd
G. virens					
Gl-3	2.04 c	12.28 c	0.51 cd	107 a	83 f
Gl-9	2.00 c	10.13 c	0.53 c	110 a	99 def
Gl-17	3.51 a	22.70 a	1.23 a	115 a	95 def
Gl-21	3.72 a	21.80 a	1.33 a	116 a	89 ef

[w] Mycelial mats of R-23 were treated with extract for 3 hr, washed, and placed in distilled water for leakage for 3 hr.
[x] Protein was assayed with Folin reagent (14), soluble carbohydrates with anthrone (15), soluble nitrogen compounds with ninhydrin (20), and electrolytes by conductance.
[y] Residual dry weight of mycelial mats of R-23 after leaching.
[z] Numbers in each column followed by same letter do not differ significantly from each other at $P = 0.05$ according to Duncan's multiple range test.

TABLE 3. Cytoplasmic leakage from hyphae of *Rhizoctonia solani* (R-23) after contact with water extracts of pathogen-infested beet seed from soils treated with germlings of *Gliocladium virens* (Gl-21)[w,x]

Extract source	Leaked materials (mg/100 mg dry wt of mycelium)[y]			
	Soluble protein	Anthrone (+)	Ninhydrin (+)	Electrolytes (μmhos)
Control (water)	0 b[z]	0 c	0 b	7 b
Beet seed + bran	0.10 b	0 c	0 b	12 b
Beet seed (R-23) + bran	0.20 b	0 c	0.15 b	15 b
Beet seed + Gl-21	0.53 a	4.54 a	0.49 a	33 a
Beet seed (R-23) + Gl-21	0.56 a	4.91 a	0.49 a	45 a

[w] Beet seed infested with *R. solani* (R-23) was incubated in soil with germlings of *G. virens* (Gl-21) for 4 days.

[x] Mycelial mats of *R. solani* were treated with extracts for 3 hr, washed, and placed in distilled water for leakage determination after 3 hr.

[y] Protein was assayed with Folin reagent (14), soluble carbohydrates with anthrone (15), soluble nitrogen compounds with ninhydrin (20), and electrolytes by conductance.

[z] Numbers in each column followed by same letter do not differ significantly from each other at $P = 0.05$ according to Duncan's multiple range test.

were produced by the biocontrol fungus after it invaded and grew within the beet seed. This was determined because extracts prepared from beet seed in association with Gl-21 germlings induced leakage from mycelial mats of R-23 after the mats were in contact with the extracts (Table 3). The extract induced leakage of all the constituents for which assays were performed (soluble protein, carbohydrate, amino acids, and salts). Activity of extracts from noninfested or pathogen-infested beet seed was similar. It only appeared necessary that germlings be present for production of effective membrane-altering materials within seed.

DISCUSSION

It is evident that strains of *Trichoderma* and *Gliocladium* are able to produce growth-inhibiting substances in bran that may also induce leakage from cells of *R. solani* with destruction of cell integrity. Toxic metabolites can be produced on agricultural waste products, such as bran, that have potential in biocontrol formulation and delivery systems (8,12,13). The most critical factor for production of materials that allow leakage appeared to be age of the biocontrol preparation. Germlings or slightly older preparations that contained actively growing hyphae produced these substances, whereas mature preparations with few active hyphae or preparations of conidia on bran did not. The influence of preparation age was also shown in another study (10) in which potency of toxin from *Hypomyces aurantius* (Pers.) Tulasne, causing vacuolation and bursting of host hyphae, decreased with preparation age. It was of major importance to demonstrate that membrane-altering materials could be produced by biocontrol fungi against a pathogen in soil. Since germlings of Gl-21 produced the substance in soil against *R. solani* (Table 3), it is reasonable to assume that the materials can be produced in a natural ecosystem. Although the substances were effective against several isolates of *R. solani*, activity against other soilborne plant pathogens remains to be determined.

No attempt was made to analyze the importance of each leaked constituent. Leakage, as a reflection of membrane damage, was demonstrated by the amount and kind of cell constituents released into distilled water as well as the reduction in weight of mycelial mats of R-23. The loss in weight of mycelium of *Pellicularia sasakii* (Shir.) S. Ito was correlated with amount of leaked protein (16). The various assays we used showed that a wide variety of compounds (nucleic acids, protein, soluble carbohydrates, amino acids, salts), similar to those in other studies, were released (3,5,7,10,16,21).

Materials in the germling extracts that induced leakage were not identified. The biochemical and biophysical properties of membrane-altering antibiotics, which include alamethicin, suzukacillin, trichotoxin, paracelsin, and gliodeliquescin, have been established. They are polypeptides of the peptaibol class with molecular masses up to 2,000 daltons (1,2). There is evidence that similar substances, as well as other antibiotics such as gliovirin and gliotoxin, are produced by various strains of *Trichoderma* and *Gliocladium* (1,2,8). No attempt was made to establish the specific mechanism by which substances in germling extracts prevent growth or induce leakage. Membrane damage has been implicated by general cell leakage and microscopic observation of cytoplasm disappearance and cell bursting (8,10,16,23). In the present study, there was an immediate and gradual increase in the extent of leakage after mats of R-23 were exposed to germling extracts for increasing periods of time. There was also a similar pattern in the leakage rate curve. These data are generally consistent with those in which toxins and fungicides affect membranes of plant pathogens (16,21).

Enzymes, some of which are readily denatured by heat, may be formed in germling preparations to disrupt pathogen membranes. Chitinase and laminarinase, which are produced by *Trichoderma* spp., may bring about biological control by lysing pathogen cell walls (4,10). However, formation and activity of the enzymes in natural soil have not been adequately demonstrated. Acetylglucosamine, a product of chitin hydrolysis, was not detected as a leakage constituent from mats of R-23 treated with germling extracts. Those enzymes that destroy the cytoplasmic membrane may be more important than cell-wall-degrading enzymes as a biocontrol mechanism. The activity of such enzymes, which include proteases, lipases, and phospholipases, has been described in general cell metabolism (22,24) but not for biocontrol fungi. In any of these studies, the key question that remains is whether a permeability change causes cell destruction or is merely another symptom of altered cell physiology.

Implication of a toxin in altering cell membranes may provide important information for future studies in biocontrol mechanisms. These naturally produced fungicides, or their analogues, may be developed and formulated for commercial use. The production of a specific, easily detected compound formed in vitro by an effective biocontrol fungus may be used as a marker to isolate or develop other potentially effective biocontrol agents. There are many intriguing and useful aspects of this research that merit additional investigation.

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