Mechanism of Lysis of Fungal Mycelia in Soil

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Michigan Agricultural Experiment Station Journal Article No. 4760. Supported in part by U.S. National Science Foundation Grant GB-7281. We thank Sarah Jane Thomas for technical assistance.

Accepted for publication 25 August 1969.

ABSTRACT

The role of autolysis in the lysis of fungal mycelia in natural soil was investigated. Beta-D-glucosidase and chitinase activity in living mycelia of *Helminthosporium victoriae, Glomerella cingulata,* and *Fusarium solani* increased rapidly during incubation on membrane filters placed on soil or sterile sand leached slowly with running water. Enzyme activation stopped when mycelium was transferred to a nutrient solution, but resumed when mycelium was returned to soil or leached sand. Activity of lytic enzymes in mycelia of the three fungi, or of the same fungus at different ages, during incubation on soil, was directly correlated with susceptibility to lysis. Several streptomycetes that caused only slight lysis of dead mycelia of the three fungi completely lysed living mycelia in buffer solutions. No antibiotic activity was detected in such cultures, nor in concentrated aqueous or organic solvent extracts of soil amended with 0.1% (dry wt basis) of mycelium of *H. victoriae* or *G. cingulata.* These data support the autolysis hypothesis for lysis of fungal mycelia in soil, and indicate that autolysis is induced by nutrient deprivation without involvement of antibiotics. Phytopathology 60:148-154.

Lysis of fungal mycelia in soil is often attributed to cell wall degrading enzymes produced by lytic microorganisms in soil (3, 12, 17). When grown in the presence of fungal mycelia, a number of soil microorganisms are able to produce enzymes such as chitinase and glucanases, that may hydrolyze major constituents of fungal cell walls. Preparations containing these enzymes have been shown to lyse dead or moribund mycelia, or cell wall preparations (4, 6, 11, 15, 17). Lysis of living mycelia by enzyme preparations also has been reported (1, 3). However, enzyme preparations may also fail to lyse mycelia (11, 12), or may hydrolyze dead but not living mycelia of the same fungi (11).

Recent evidence from our laboratory suggested that soil mycolysis may be caused by autolysis (lysis by enzymes present in the mycelium itself) (10). Living mycelia of several fungi were lysed partially or completely when separated from soil by membrane filters which excluded microorganisms and enzymes from soil. Lysis of mycelia of *Glomerella cingulata* and *Helminthosporium victoriae* was induced in a model system combining starvation conditions and any of several antifungal antibiotics without the presence of soil or other microorganisms.

In this report, the activity of autolytic enzymes in mycelia during lysis in soil was studied. The factors inducing autolysis of mycelia in soil were also reinvestigated.

MATERIALS AND METHODS.—*Microorganisms.*—Actinomycetes capable of causing lysis were isolated from soil. Ten ml of molten water agar containing a diluted soil suspension were placed on 3-day-old cultures of *G. cingulata* growing on 0.5% peptone agar in petri dishes. After 12 days' incubation at 26°C, colonies producing lytic zones were isolated. All isolates were of the genus *Streptomyces.*

Hyphae of *H. victoriae* Meehan & Murphy, *G. cingulata* (Ston.) Spauld. & Schrenk, and *Fusarium solani* f. sp. *pisi* (F.R. Jones) Snyd. & Hans. were obtained by germinating conidia in potato-dextrose broth (PDB) at 26°C. The hyphae were washed twice with sterile water by centrifugation before use. When dead hyphae were to be used, they were killed by exposure to 100°C for 5 min.

Lysis assays.—Conover loam topsoil from the Michigan State Univ. farm was passed through a No. 10 mesh sieve, adjusted to about 25% moisture, and stored in polyethylene containers. The soil has the following characteristics: pH, 6.7; organic matter, 3.8%; water-holding capacity, 42.7%; clay, 7.5%; silt, 42.8%; and sand, 49.7%. Direct assays for lysis followed the method of Lingappa & Lockwood (9). Indirect assays for lysis were done by incubating hyphae on Pellicon membranes (Millipore Corp.) placed on soil. These membranes exclude compounds of molecular weight greater than approximately 1,000, and were not degraded after 3 months' incubation in soil. After incubation, the membrane was removed from soil, and the surface facing the soil was washed. Hyphae were stained with rose bengal, and the membrane was dried at 80°C for 10 min and mounted in immersion oil to make it transparent. Amount of lysis was estimated on a 0-6 scale: 0 = no lysis; 1 = 1-10% of hyphae lysed; 2 = 10-30% of hyphae lysed; 3 = 30-70% of hyphae lysed; 4 = 70-90% of hyphae lysed; 5 = 90-99% of hyphae lysed; 6 = 100% of hyphae lysed. Hyphae recovered immediately following addition to soil served as controls.

Enzyme assays.—Enzyme extracts were prepared by grinding mycelium in 4 ml 0.1 M sodium acetate buffer (pH 5.4) first in a mortar, then in a tissue homogenizer. The homogenate (0.4 ml) was incubated for 5 min with 3.6 ml *p*-nitrophenyl-β-D-glucopyranoside (0.5 mg/ml) at 30°C for determination of β-D-glucosidase (18), or with *p*-nitrophenyl-N-acetyl-β-D-glucosaminide (0.15 mg/ml) at 38°C for determination of chitinase (19). After incubation, the color of the liberated *p*-nitrophenol was developed with 0.5 ml of 1.0 M Tris[tris (hydroxymethyl) amino methane] (pH 9.8). The mix-

TABLE 1. Lysis of fungal mycelia on soil and in model systems

Treatment	Lysis rating[a]					
	Helminthosporium victoriae		*Glomerella cingulata*		*Fusarium solani*	
	Living	Dead	Living	Dead	Living	Dead
Directly on soil	5	2	5	3	5	2
Pellicon membrane on soil	4	0	3	0	2	0
Dripping water[b]	4	0	3	0	2	0
Leached sand[c]	4	0	3	0	2	0

[a] Lysis was estimated on a 0-6 scale: 0 = no lysis, 1 = 1-10%, 2 = 10-30%, 3 = 30-70%, 4 = 70-90%, 5 = 90-99%, and 6 = 100% of hyphae lysed.

[b] Mycelia placed on a Millipore filter in a filter holder were continuously leached with slowly dripping distilled water or buffer solution.

[c] Mycelia on Millipore filters were incubated on washed sea sand continuously leached with slowly flowing water.

ture was centrifuged at 27,000 *g* for 5 min, and absorbance of the supernatant was determined in a colorimeter (Bausch & Lomb Spectronic 20) at 400 mμ. A standard curve was made with solutions containing 10, 20, and 30 μM *p*-nitrophenol. One unit of enzyme was defined as that amount which would liberate 1 μM *p*-nitrophenol in 1 min.

Determination of N-acetylglucosamine and carbohydrates.—*N*-acetylglucosamine was determined by the method of Reissig et al. (16). A standard curve was made from solutions containing 12, 24, and 48 μg of N-acetylglucosamine/ml. Carbohydrates were determined by the anthrone method (14), using glucose at concentrations of 20, 40, and 80 μg/ml as a standard.

RESULTS.—*Lysis of fungal hyphae on Pellicon membranes placed on soil.*—Living or dead hyphae of the three test fungi were placed on Pellicon membranes on soil. Hyphae incubated directly on soil were used for comparison. After 4 days' incubation at 26°C, lysis of living mycelium was as follows: 70-90% for *H. victoriae;* 30-70% for *G. cingulata;* and 10-30% for *F. solani* f. sp. *pisi* (Table 1). Although the rate of lysis on membranes was slower than that occurring directly on soil, the characteristics of lysis in the two treatments were similar. Since enzymes are unable to pass through Pellicon membranes, lysis of mycelia on soil was apparently due to self-digestion. This conclusion was supported by the failure of dead mycelia to lyse when incubated on membranes on soil, and by faster and more complete lysis of living than of dead mycelia incubated directly on soil. These observations confirmed results previously obtained by Lloyd & Lockwood (10) with conventional membrane filters.

Lysis of fungal hyphae by streptomycetes.—Conidia of *H. victoriae, G. cingulata,* and *F. solani* f. sp. *pisi* were germinated in PDB for 24 hr at 26°C. Washed mycelia were then suspended in 0.05 M phosphate buffer (pH 6.9). Streptomycete spores from agar slants were suspended in sterile distilled water. An 8-ml mycelial suspension was incubated with 2 ml streptomycete suspension in a sterilized 50-ml Erlenmeyer flask for 4 days under shaking conditions. After incubation, the mycelium was transferred to a glass slide, stained with rose bengal, and observed microscopically. Mycelial suspensions incubated without streptomycetes were used as controls.

Each of the four streptomycetes caused nearly complete lysis of living mycelia, but induced only slight lysis of dead mycelia (Table 2). Ninety-99% of living mycelia of *H. victoriae* and *G. cingulata,* and 30-70% of that of *F. solani* f. sp. *pisi* were lysed, as compared with only 1-30% lysis of dead mycelia. In the absence of streptomycetes only 0-10% of the living mycelia lysed, and dead mycelia remained intact. The results resembled those in soil, in that living mycelia were lysed more readily than were dead mycelia.

Antibiotic production during lysis of fungal mycelia.—The results of Lloyd & Lockwood (10) indicated that nutrient deficiency and antibiotics were jointly involved in the induction of autolysis of fungal mycelia in soil. They showed antibiotic activity in extracts of soil after amendment with mycelium of *G. cingulata* in three of five tests. We reinvestigated the possibility that antibiotics are produced by microbial growth on fungal mycelia, using the mixed cultures of fungi and streptomycetes described in the previous section and natural soil amended with fungal mycelia. After 4 days' incubation, the mixed cultures of fungi and streptomycetes were ground in a mortar, and the homogenates were sterilized by passage through Millipore filters (0.22 μ). Spores of the same fungus used in the incubation mix-

TABLE 2. Lysis of fungal mycelia by four streptomycete isolates in buffer solutions[a]

Fungus	Mean lysis rating[b]		Mean germination, %[c]	
	Living	Dead	Filtrates	Water
Helminthosporium victoriae	5	1-2	99-100	93
Glomerella cingulata	5	1-2	78-97	8
Fusarium solani	3	1	97-100	55

[a] Each of four streptomycetes was incubated with each of three fungi for 4 days in 0.05 M phosphate buffer (pH 6.9).

[b] Lysis was estimated on a 0-6 scale: 0 = no lysis, 1 = 1-10%, 2 = 10-30%, 3 = 30-70%, 4 = 70-90%, 5 = 90-99%, and 6 = 100% of hyphae used.

[c] Mixed culture of streptomycete and living fungus was ground, and passed through a Millipore filter (0.22 μ). Conidia of the same fungus were germinated in the filtrate and in water.

ture were germinated in the filtrates. There was no evidence for the presence of antibiotics (Table 2). In every case more spores germinated in filtrates than in the water controls.

Antibiotic production by soil microorganisms during lysis of mycelia in natural soil was investigated in three ways: (i) One hundred g soil containing 0.1% mycelium of *G. cingulata,* on the basis of dry wt, was incubated at 26°C for 2 or 4 days. The soil was then shaken for 30 min with 50 ml distilled water, and the suspension was centrifuged at 12,000 *g* for 5 min. The supernatant was sterilized by passage through Millipore filters (0.22 μ). The pellet was extracted with 50 ml of a mixture of chloroform and methanol (1:1, v/v). The solvent extract was passed through Whatman No. 1 filter paper, and the filtrate was taken to dryness in a flash evaporator at 30°C. The dried material was taken up in 10 ml distilled water, and the solution was sterilized by Millipore filtration. Soil without mycelium was also extracted using the same procedure. (ii) Two-tenths-g wet mycelium of *G. cingulata* was incubated on a Millipore filter on 20 g soil at 26°C for 2 or 4 days. The mycelium was then ground with 10 ml distilled water in a mortar, and the extract was sterilized by Millipore filtration. (iii) Two-tenths-g samples of wet mycelium of *G. cingulata* or *H. victoriae* were incubated directly on soil for 2 or 4 days at 26°C. After incubation, the mycelium was scraped off the soil. Separate samples were extracted in each of three ways: (i) The mycelium was ground with 1 ml distilled water in a mortar and the extract was sterilized by Millipore filtration. (ii) The mycelium was extracted with 10 ml of chloroform:methanol mixture. The extract was passed through a filter paper, and the filtrate was evaporated to dryness. The dried material was suspended in 1 ml sterile distilled water. (iii) The mycelium was extracted with 4 ml chloroform:methanol mixture. The extract was centrifuged at 15,000 *g* for 5 min, and the supernatant was evaporated to dryness, aseptically, in a petri dish. The dried material was resuspended in 1 ml sterile distilled water. The extracts were used for germination tests with conidia of the same fungus as that used for amendment. Duplicate samples were used, and the experiments were repeated.

Conidia of *G. cingulata* or *H. victoriae* germinated 80-100% in all the extracts. There was no evidence for the presence of antibiotics in nonamended soil, or for the production of antibiotics during lysis of mycelia in soil.

Lysis of fungal mycelia in model systems.—If autolysis of fungal mycelia in soil is induced by nutrient deprivation alone, it should be possible to induce lysis by exposing mycelia to systems which provide for movement of nutrients away from the fungus. To test this possibility, the leaching system designed for soil fungistasis studies (8) was used. Living and dead mycelia of *H. victoriae, G. cingulata,* and *F. solani* f. sp. *pisi* were placed on separate Millipore filters (0.22 μ) in fritted glass filter holders, and were leached aseptically by dripping distilled water or 0.01 M phosphate buffer (pH 6.9) onto the mycelia at the rate of 10-30 ml/hr. Another model system (Fig. 1) which resembles the conditions of soil more closely was also designed. A large petri dish (150 × 20 mm) was fitted with an inlet tube at one side of the cover and an outlet tube at the bottom on the opposite side. Mycelia of *H. victoria, G. cingulata,* and *F. solani* f. sp. *pisi* were placed on separate Millipore filters on washed sea sand in the petri dish. Distilled water in a separatory funnel was dripped onto the sand through the inlet at the rate of 10-30 ml/hr. The water moved slowly through the sand

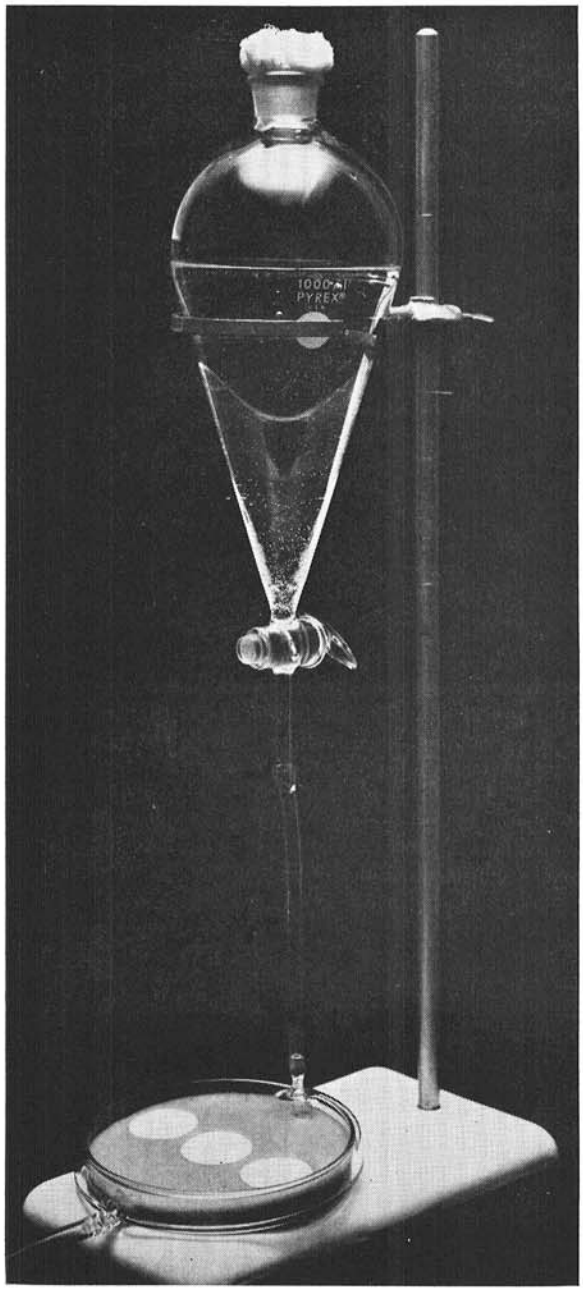


Fig. 1. Model system used to induce autolysis of fungal mycelia. Mycelia were placed on membrane filters on washed sand in the petri dish. Water from the separatory funnel dripped slowly (10-30 ml/hr) onto sand at one side of the dish and drained from the opposite side.

and drained from the outlet. By this means, mycelia on Millipore filters on sand were continuously exposed to a steep diffusion gradient. The apparatus was sterilized before use, and remained uncontaminated during the experiments.

Living mycelia lysed when they were exposed to the conditions of either model system (Table 1). After 4 days' incubation, mycelia of *H. victoriae, G. cingulata,* and *F. solani* f. sp. *pisi* lysed 70-90%, 30-70%, and 10-30%, respectively. The rate and character of lysis in the leaching systems were similar to those on Pellicon membranes on soil. Living mycelia incubated on moist sand without flowing water lysed 1-10%. Dead mycelia showed no lysis on either model system.

Activation of autolytic enzymes in fungal mycelia during lysis.—If lysis of fungal mycelia in soil is due to self-digestion, increase in autolytic enzyme activity should occur during lysis. The activities of chitinase and β-D-glucosidase in mycelia during lysis were studied, since chitin and glucans are two of the important components of fungal cell walls. Three-day-old mycelia of *H. victoriae, G. cingulata,* and *F. solani* f. sp. *pisi* grown in PDB were washed three times with sterile distilled water. Duplicate mycelial samples of 0.2-0.4 g each, for each fungus, were placed on Millipore filters on natural soil or on Millipore filters on leached sand. Mycelium, incubated in 5 ml PDB in a petri dish, was used as a control. After 24-hr incubation, chitinase and β-D-glucosidase activities in the mycelium were determined. Additional triplicate samples were used for dry wt determinations.

Enzyme activity increased rapidly in mycelia incubated on natural soil (Fig. 2-A) or on leached sand (Fig. 2-B). For example, activity of β-D-glucosidase after one day's incubation on soil or leached sand, respectively, increased 16- and 15-fold for *H. victoriae,* 8- and 4-fold for *G. cingulata,* and 5- and 1.4-fold for *F. solani* f. sp. *pisi.* Initial levels of β-D-glucosidase were 0.7-1.2 units/g dry mycelium. Corresponding increases in chitinase activities were 17- and 11-fold, 14- and 11-fold, and 2.6- and 1.7-fold. Initial levels were 1.5-2.0 units. The activity of enzymes detected in the three fungi were directly correlated with their rates of lysis (Fig. 2-A, B). Chitinase and β-D-glucosidase activities increased only slightly in PDB.

To determine whether increased chitinase and β-D-glucosidase activities in mycelia incubated on Millipore filters on soil could have originated from soil rather than from the mycelia, mycelium of *G. cingulata* was incubated on a Millipore filter on 20 g natural soil for 1 day. This soil or control soil was then shaken with 5 ml distilled water for 30 min. The soil suspension was centrifuged at 27,000 *g* for 5 min, and the supernatant was passed through a Millipore filter. Neither enzyme was detected in any of the filtrates.

The effect of time of incubation on increased activity of autolytic enzymes in mycelia was studied. Mycelia of *G. cingulata* were placed on Millipore filters (22 μ, 37-mm diam) and incubated on soil, leached sand, or in PDB. After different periods of incubation, chitinase and β-D-glucosidase were determined. Both chitinase and β-D-glucosidase in the mycelium incubated on soil or leached sand increased rapidly for 2 days (Fig. 3-A, B). Thereafter, activity of both enzymes either increased more slowly or decreased. Only small increases occurred in mycelium incubated in PDB.

The effect of alternate incubation in deprived and

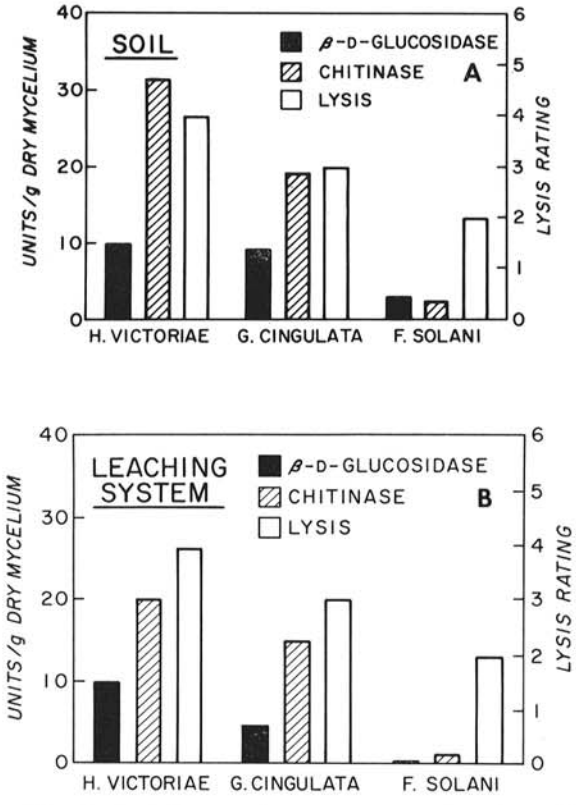


Fig. 2. Activities of β-D-glucosidase and chitinase in mycelia, and lysis of mycelia, of three fungi after 4 days' incubation on Pellicon membranes on **A)** soil, or **B)** leached sand.

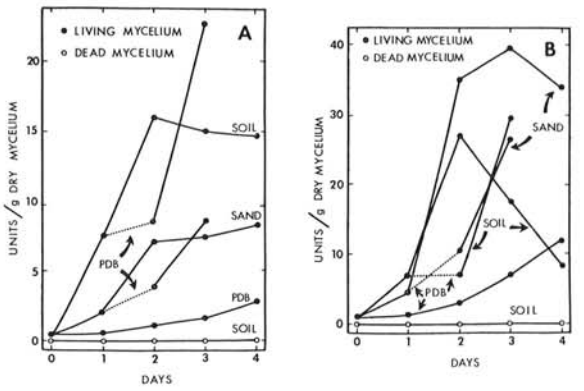


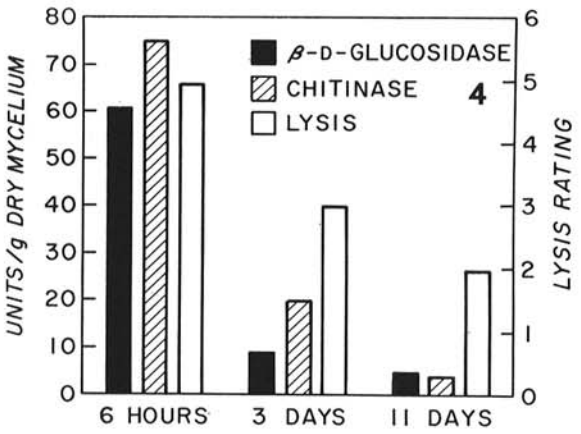
Fig. 3. Effect on autolytic enzyme activities of incubating mycelia of *Glomerella cingulata* on membrane filters on soil, leached sand, or potato-dextrose broth (PDB); and of alternate incubation for 1 day each on soil or leached sand, then on PDB, then again on soil or leached sand. Dead mycelium was incubated on membrane filters on soil as a control. **A)** Beta-D-glucosidase, **B)** chitinase.

nutrient conditions on enzyme induction was studied. Mycelium on a Millipore filter (37-mm diam) was placed on a larger filter (47-mm diam) which was placed on soil to prevent contamination of the upper filter. During 1 day's incubation on soil or leached sand, activities of β-D-glucosidase and chitinase increased (Fig. 3-A, B). Activation stopped or decreased markedly when the mycelium was transferred to PDB for 1 day, but reactivation occurred rapidly when the mycelium was returned to soil or leached sand. Dead mycelium incubated on Millipore filters on soil showed no enzyme activity during 4 days' incubation.

The effect of age of mycelium on susceptibility to lysis and enzyme activity was determined. Three- and 11-day-old mycelia of *H. victoriae* were triturated for 1 min in a Sorvall Omni-Mixer at 3,000 rpm, and washed twice with sterile distilled water. Younger hyphae were obtained by germinating conidia of *H. victoriae* in PDB for 6 hr. Hyphae were washed twice before use. Lysis was determined after diluted hyphal suspensions had been incubated in direct contact with smoothed soil surface for 4 days. Enzyme activities were determined, using approximately equal portions (about 0.3 g) of wet mycelia also incubated directly on soil. After 24-hr incubation, mycelia were removed from soil and assayed for chitinase and β-D-glucosidase activities. Sensitivity to lysis and levels of chitinase and β-D-glucosidase activity were greatest in young mycelia (Fig. 4).

Lytic activity of induced chitinase and β-D-glucosidase.—Mycelium of *G. cingulata* was incubated on Millipore filters placed on soil or leached sand. After 24 hr, the mycelium was ground with 4 ml 0.1 M sodium acetate buffer (pH 5.4), first in a mortar, then in a tissue homogenizer. One ml homogenate was incubated with 1 ml sodium acetate buffer at 38°C for 8 hr. The mixture was then centrifuged at 12,000 *g* for 5 min, and the supernatant was passed through a Millipore filter. The filtrate was assayed for *N*-acetyl-glucosamine and soluble carbohydrates. The mycelium incubated on soil released 38 μg carbohydrates and 1.7 μg *N*-acetylglucosamine/g dry mycelium, and mycelium incubated on leached sand released 26 μg carbohydrates and 1.3 μg *N*-acetylglucosamine/g dry mycelium. Thus, the enzymes activated during exposure of mycelium to conditions of nutrient deprivation are capable of lysing cell-wall components.

Relation between death and autolysis of fungal mycelia.—Conidia of *H. victoriae* were germinated in PDB for 6 hr, washed twice with sterile distilled water, and applied directly to soil. After different periods of incubation, hyphae in two samples were stained and recovered, and the number of lysed cells in 20 hyphae was determined. Other samples were used to determine the viability of lysed cells by adding 0.2 ml PDB to soil containing the hyphae. After incubation for 6 hr, hyphae were stained with rose bengal and recovered. Cells which neither stained nor produced new germ tubes were considered dead. Three-day-old mycelium of *H. victoriae* incubated directly on soil was used to determine enzyme activity after different periods of incubation.



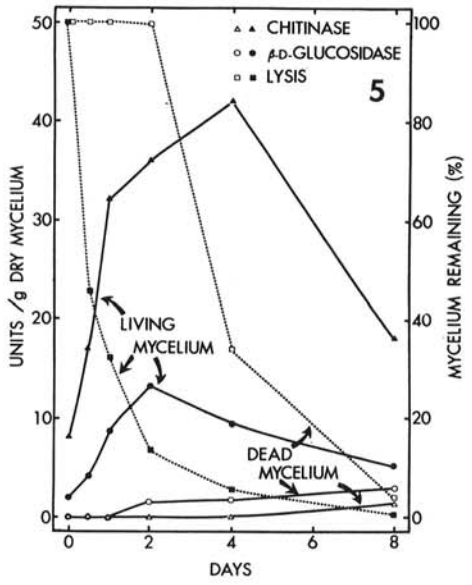


Fig. 4-5. **4)** Relation between age, susceptibility to lysis, and activities of autolytic enzymes in living mycelium of *Helminthosporium victoriae* incubated directly on soil. **5)** Relation between rate of lysis and activities of autolytic and heterolytic enzymes in living and dead mycelia of *H. victoriae* incubated directly on soil.

Death of cells and activation of autolytic enzymes occurred very rapidly (Fig. 5). Within 24 hr, about 65% of the cells in the hyphae were dead, and both chitinase and β-D-glucosidase activities had increased more than 4-fold. Lysis of dead mycelia did not commence until after the 2nd day; a low level of β-D-glucosidase activity was detected in dead mycelia after 2 days, and chitinase was not detected until between 4 and 8 days.

DISCUSSION.—The cause of the lysis of fungal mycelia in soil is a fundamental and controversial question. There is support for both heterolytic and autolytic mechanisms. Evidence supporting the heterolytic hypothesis is based mainly on the fact that lytic micro-

organisms may produce enzymes capable of degrading fungal mycelia (1, 3, 4, 6, 15, 17). However, there are exceptions; several bacteria which did not produce chitinase were able to lyse fungal mycelia in agar media or sterilized soil (10), and conversely, both chitinase and β-1,3-glucanase were produced by a bacterium and a fungus which were nonlytic (12). Evidence for production of cell-wall degrading enzymes and their role in lysis of fungi in soil is scant. Jones & Webley (7) detected glucanase activity in autoclaved cell walls of *Fusarium culmorum* and a sterile fungus after 14 days' incubation in soil. We were also able to detect low levels of β-D-glucosidase and chitinase activity in dead mycelium of *H. victoriae* after 2 and 4 days' incubation, respectively, in soil (Fig. 5). However, since at least 65% of the cells of the living mycelium of *H. victoriae* had lysed within 24 hr in soil, it is unlikely that lysis in this case could be due to enzymes produced by other microorganisms in soil. Attempts to extract lytic factors from soil were not successful (2).

The autolysis hypothesis is supported by our results and those described elsewhere (10), and may be summarized as follows: (i) Living mycelia of several fungi were completely or partially lysed when soil was separated from fungal mycelia by Millipore filters (10) or by Pellicon membranes with pores small enough to exclude enzymes from soil. However, dead mycelia remained intact. (ii) Living mycelia characteristically lysed faster or more completely than dead mycelia when incubated in mixed liquid cultures with actinomycetes, in agar cultures with actinomycetes (11), and on natural or actinomycete-infested sterilized soil (10). (iii) Lysis of living mycelia, characteristic of that occurring in soil, occurred under axenic conditions in model systems which imposed deprivation conditions on the fungus. (iv) Chitinase and β-D-glucosidase in fungal mycelium increased very rapidly during incubation on soil or leached sand. The activated enzymes were able to hydrolyze cell-wall constituents of the mycelium producing them. The two enzymes studied in the present work, though activated early following exposure to soil or leached sand, may or may not be the enzymes responsible for death of hyphal cells. Other autolytic enzymes activated by the same conditions may be the direct cause of death. (v) Decreased activities of chitinase and β-D-glucosidase were correlated with a decreased rate of lysis of mycelium of *G. cingulata* as mycelia aged. A similar correlation was shown between activity of these enzymes and the relative susceptibility to lysis of the three fungi tested. Since enzyme activity was expressed on the basis of dry wt of mycelia, the apparently lower enzyme activities in older mycelia might be an artifact due to increased deposition of solid material in the wall rather than to decreased enzyme activity. However, it seems unlikely that the large differences shown would be accounted for entirely by increased dry wt of mycelia.

Apparently little is known concerning the activity and induction of autolytic enzymes in fungi. Mitchell & Sabar (13) detected strong autolytic glucanase and protease activities in cell-wall preparations of *Pythium* spp. prepared by ultrasonic treatment, and Jones & Webley (7) reported an active autolytic glucanase in hyphae of *Fusarium culmorum* and an unidentified sterile fungus made into aggregates with kaolinite and incubated on soil.

The induction of autolysis by nutrient deprivation appears to be an ecologically reasonable model. The flowing water in the model systems imposes on the fungus, by physical means, a nutritionally deprived environment which is presumably similar to that imposed in soil by microbial activity. Lysis of fungi in soil (10), as is well known in pure cultures, characteristically occurs under conditions of deficient or declining nutrient levels.

In the study of Lloyd & Lockwood (10), mycelia incubated on dialysis tubing containing flowing water were lysed completely only when an antibiotic was applied to the hyphae. However, the dialysis system apparently was not as efficient in removing nutrients as the model systems used in the present study. This was indicated by the very slow diffusion of food colors from agar discs incubated on dialysis tubing (Ko & Lockwood, *unpublished data*). Lloyd & Lockwood (10) also demonstrated antibiotic activity in some extracts of soil amended with 1% of mycelium. However, in the present work, using soil amended with 0.1% of mycelium, we were not able to detect antibiotic activity in extracts of soil or of mycelia recovered from soil. Moreover, several actinomycetes caused complete lysis of living mycelium in buffer solutions without producing detectable antibiotic substances. Since mycelia never exist in large quantities in nature, it is unlikely that antibiotics were produced during lysis of mycelia in soil. The role of antibiotics in lysis was also supported by a correlation between sizes of lytic and inhibition zones produced by streptomycetes (11). However, recent work (5) indicated that about half of 18 lytic streptomycetes tested produced inhibition zones against fungi by depleting the agar of nutrients rather than by producing antibiotics. Zones which contained antibiotics also had reduced nutrient levels. Therefore, the nutrient status of agar adjacent to streptomycete colonies may be important in determining the existence or size of inhibition zones.

In summary, our results indicate that when fungal mycelia are exposed to soil, autolytic enzymes in mycelia are activated due to nutrient deprivation imposed by microbial activity. The activated enzymes dissolve cell constituents, leading to death. Enzyme production by other microorganisms may be a factor in degrading the remaining lysed mycelia at a late stage of soil mycolysis. Thus, nutrient deprivation may be responsible for both soil fungistasis (8) and mycolysis.

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