Processes of Sporulation in Alternaria solani and their Response to Metabolic Inhibitors

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ABSTRACT

Hyphae and conidiophores of *Alternaria solani* are both filaments. In that sense, both conidiophores and spores arise as buds from filaments. Both budding processes require oxygen and are inhibited by carbon dioxide. They are highly sensitive to azide, dinitrophenol, and thiol reagents, but not to cyanide nor fluoride. Acridine and 2 phenylcarbamates as well as several phenoxyacetic acids and other inhibitors of glycolate oxidase were toxic to both budding processes. These results suggest that respiration of budding involves an iron-flavin terminal system that has a high affinity for oxygen. Budding may require ATP and thiols. The oxidation of glycolic acid and the subsequent utilization of glyoxylic acid appear essential to budding. Budding seems to involve synthesis of nucleic acids and proteins. Oligomycin, 8-azaguanine, and 2-thio-6-oxypurine inhibit formation of conidiophores but not spores. Apparently, these differences in budding response between a hypha and a conidiophore reflect slight differences between the two filaments. Budding, therefore, can be a target at which to direct a search for new fungicides of high selectivity.

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Spores are a very important form of inoculum produced by many pathogenic fungi. Thus, factors that hinder sporulation may be useful in disease control. Moreover, to combat disease with selective inhibition of sporulation may prove less hazardous than with fungicides.

Generally, sporulation involves specific physiological processes. Its oxygen and carbon dioxide requirements differ from those of mycelial growth, and precise requirements vary with fungal species and with light (4, 16). Respiration during sporulation of *Aspergillus niger* may proceed by way of glycolysis and the citric acid cycle, whereas respiration during mycelial growth may involve part of glycolysis (3). However, respiration may shift from the citric acid cycle to the glyoxylate cycle when acetate is the carbon source (21). The oxidation of glycolate and the utilization of glyoxylate appear to be involved in sporulation of *Alternaria solani* (10, 15). Chemical analysis of conidia and mycelium has revealed that fatty acid ketones are produced by spores of *Penicillium roquefortii* but not by mycelia (7). Conidia of *Ophiostoma multiannulatum* contain more DNA components than do hyphae (22).

In the genus *Alternaria*, the process of sporulation can be separated from that of mycelial growth, and then the two steps of sporulation, formation of conidiophore (hereinafter called "stalk" for brevity) and initiation of conidium can be examined independently of each other (2, 11, 13, 25). Near ultraviolet irradiation, removal of carbon source, injury to mycelium, and moisture encourage the mycelium to produce stalks. The initiation of conidia at the terminals of the stalks is induced by cool temperature or the absence of visible light. The environmental parameters of stalk and spore formation in *A. solani* have been given previously (23).

In this report we describe the development of a conidium in *Alternaria solani* and examine some aspects of metabolism required for growth of stalk and spore. We examined metabolism of sporulation by subjecting the fungus to mixtures of oxygen and carbon dioxide and to different metabolic inhibitors.

MATERIALS AND METHODS.—The sporulating process of *Alternaria solani* (Ell. & G. Martin) L. R. Jones & Grout. was induced according to the techniques described previously (15). Stalks were grown on filter paper from hyphal fragments that had been placed in 0.02 M phosphate (pH 6.3) on filter paper and held in cool-white fluorescent light, 50-75 ft-c, at 23 C. Samples were removed at intervals to determine the progress of spore development. We observed the production of stalks by folding the filter paper culture and placing the edge between a microscope slide and cover glass. Stalks extending in a horizontal plane from the edge of the paper were photographed under 430 times magnification.

We exposed the fungus to various atmospheres by placing small strips (1 × 5 cm) of filter paper culture in oxidation tubes and flushing the tubes 5 times with gas mixtures. For a dosage series of oxygen, air was diluted with nitrogen (v/v). We removed traces of oxygen by bubbling nitrogen through solutions of chromous chloride. Carbon dioxide was diluted with air (v/v) to obtain a dosage series. We determined the effect of gases on stalk formation by placing hyphal fragments in the test atmosphere and recording the number of stalks developing in 2 days. We determined the effect of oxygen or carbon dioxide on spore initiation by gasing stalks at the time of inducing conidial formation. All experiments were repeated one or more times.

We applied inhibitors by dipping filter papers into water or acetone solutions of test chemicals and evaporating the solvent from the papers. In tests on

formation of stalks, hyphal fragments were placed directly on the chemically treated paper. In tests on formation of spores, the treated paper was placed beneath the paper bearing a culture of 31-hr-old stalks at the time of spore induction (15). This method of testing may not detect the activity of cationic substances that may bind to the filter paper or of volatile substances that may escape with the evaporating solvent from the filter paper.

The number of stalks formed in 2 days was determined by a counting of 10 microscopic fields (area per field = 0.74 mm^2) at 100 times magnification for each of two cultures/treatment. The average yield in untreated cultures was about 12 stalks/field. After determination of stalk yield, sporulation was induced and then examined the following day to verify that the stalks were still alive. Finally, we stained cultures with trypan blue in lactophenol to detect mycelial growth of original fragments of hypha to verify that concentrations of chemical that inhibited stalk formation did permit mycelial growth.

The effects of chemicals on spore initiation were determined by examining 100 stalks/treatment under 100 times magnification 16 hr after the time of treatment, and the percentage of stalks lacking conidia was recorded. Sporulation of untreated stalks was always above 95%. We also noted whether the barren stalk was collapsed or turgid, growing or not growing.

RESULTS.—*Formation of stalks.*—A stalk begins as a bud on the side of a hypha and elongates in a filamentous fashion to a length of 15-17 cells (Fig. 1).

Effect of gas mixture.—The removal of oxygen had little effect on the number of stalks formed. The number of stalks growing in the presence of various oxygen tensions was 13, 12, 8, 12, and 11 for oxygen concentrations of 20, 10, 5, 2.5, and 1.25%, respectively. Even when the oxidation tubes containing hyphal fragments were flushed 5 times with nitrogen, formation of stalks was still at 10. In the evacuating procedure, we observed minute bubbles to cling to fibrils of the moist paper. Apparently, this residual oxygen in the culture was sufficient for the fungus to grow stalks. Cultures from all treatments formed stalks when placed in air in the dark at 23 C.

Air enriched with carbon dioxide inhibited formation of stalks. The numbers per field were 14, 9, 8, 6, 2, and 0 for 0, 1, 3, 10, 20, and 50% carbon dioxide enrichment in air, respectively. The ED_{50} value was 4.6% carbon dioxide. Some stalks forming in carbon dioxide atmospheres of 3% or greater reverted to fine aerial hyphae after growing several cells. Others formed spores when placed in air and darkness at 23 C.

However, if stalks were formed in air enriched with carbon dioxide and were retained in that atmosphere during the spore induction period, they failed to form spores. Evidently, the physiology of stalks differs when they are formed in air rather than in air enriched with carbon dioxide. Partial inhibition of hyphal growth occurred in 10 and 20% carbon dioxide atmospheres.

When the carbon dioxide was diluted with nitrogen, the ED_{50} value for inhibiting formation of stalks was 3.0% carbon dioxide. Thus, the reduction of ED_{50} values from 4.6 to 3.0 with the extreme reduction in oxygen tension suggests that oxygen tension has little effect on the inhibitory effects of carbon dioxide.

Effect of respiratory inhibitors.—The dosage of toxicant required to inhibit stalk formation by 50% is presented in Table 1. The lethal dosage is indicated

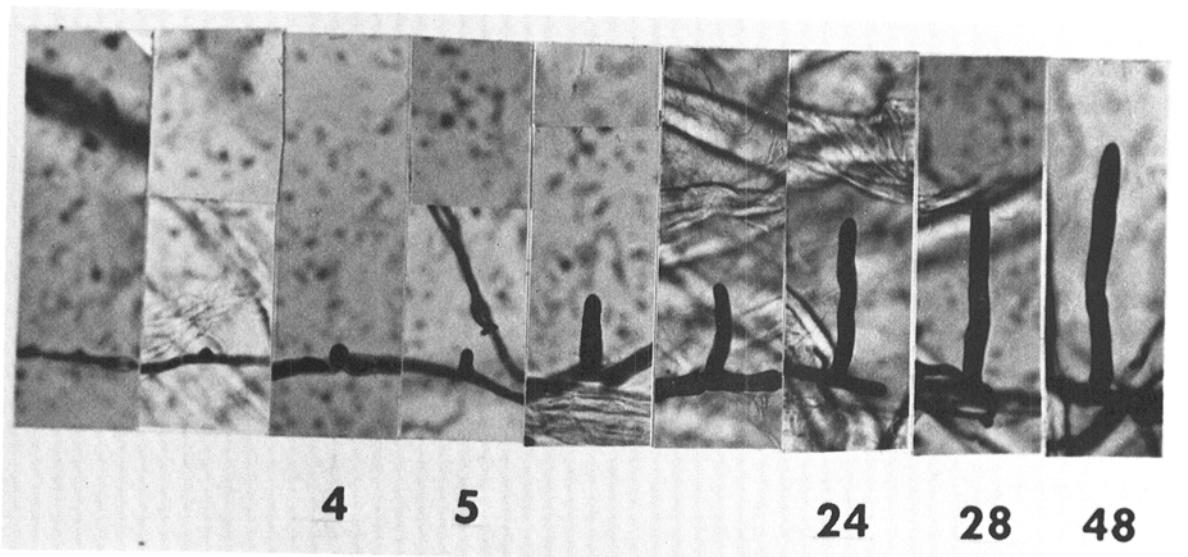

Fig. 1. The development of a stalk of *Alternaria solani*. Hyphal fragments were washed, suspended in phosphate buffer (0.02 M, pH 6.3), and applied to filter paper for incubation in light at 23 C. Cultures were examined at the hour indicated.

TABLE 1. Toxicity of respiratory inhibitors to sporulation of *Alternaria solani*

Inhibitor	ED_{50} values (mM)			Toxic ratio[a]
	Stalk formation	Conidial formation	Stalk collapse	
Sodium cyanide	9.80	16.10	>20.40	0.61
Sodium azide	0.66	0.56	>15.40	1.18
Sodium fluoride	5.96	>20.00	>100.00	<0.30
Potassium iodide	0.97	3.00	>6.00	0.32
Sodium diethyldithiocarbamate	<0.01[b]	0.93	>4.45	<0.01
N-Ethylmaleimide	0.07	0.13	0.45	0.54
Iodoacetic acid	0.01	0.47	3.87	0.02
Cobaltous chloride	0.32	1.09	>4.20	0.29
Nickelous chloride	0.13	0.30	>4.20	0.43
Oligomycin	<0.03	>2.30	>2.30	<0.01
2,4-Dinitrophenol	0.64	0.49	1.14	1.31
2,4-Dinitro-6-nonylphenol	<0.01[c]	0.01	0.21	0.80
Crotonic acid	6.40	4.08	10.90	1.57
Undecanoic acid	0.32	0.67	1.20	0.48
10-Undecenoic acid	0.54	0.68	0.72	0.79
Dicoumarol	>3.02	>3.02	>3.02	
Alloxan	>7.04	>7.04	>7.04	

[a] ED_{50} for stalk formation/ED_{50} for conidial formation.
[b] ED_{50} value = 0.005 mM.
[c] ED_{50} value = 0.008 mM.

by the dosage of toxicant required to collapse half the number of stalks in the spore assay. Among the inhibitors of terminal respiration, azide was very effective against stalk formation at concentrations of less than 1/25 of the lethal dosage. Cyanide did not affect stalk formation. The inaction of cyanide was not due to the escape of hydrogen cyanide from a culture, because sealing of the culture dish to entrap potential gases did not change the results. Fluoride and iodide slightly reduced formation of stalks.

All of the thiol reagents prevented formation of stalks at concentrations that were not lethal to hyphae.

Oligomycin, an inhibitor of oxidative phosphorylation, reduced stalk formation.

Compounds known to uncouple phosphorylation from respiration gave varying results. Dinitrophenol (DNP) was more effective against stalk formation than against hyphal growth. The compound, 6-nonyl-2,4-dinitrophenol, was highly effective against stalk formation. The addition of the 9-carbon chain to DNP, which presumably increases permeability by its increased lipophilicity, also increased toxicity. Crotonic and undecanoic acids were slightly inhibitory to stalk formation. However, the unsaturated undecanoic acid was more toxic to

TABLE 2. Toxicity of inhibitors of glycolate and glyoxylate metabolism to sporulation of *Alternaria solani*

Inhibitor	ED_{50} values (mM)			Toxic ratio[a]
	Stalk formation	Conidial formation	Stalk collapse	
Phenoxyacetic acids				
4-Nitro-	0.56	10.00	>10.00	<0.06
2,4-Dichloro-	2.94	3.17	4.62	0.93
2,4,6-Trichloro-	0.08	0.23	1.37	0.35
2,3,5,6-Tetrachloro-	2.71	1.81	3.56	1.49
Pentachloro-	0.19	0.28	1.19	0.67
4-Chloro-2-cyclohexyl-	0.45	0.93	1.20	0.48
Hexachloro-2-propanol	0.06	0.13	0.93	0.46
α-(Trichloromethyl)benzyl alcohol	0.23	0.54	2.14	0.43
α-(Trichloromethyl)2-pyridine ethanol	0.92	1.84	>4.20	0.50
1,1,1-Trichloro-3-nitro-2-propyl carbanilate	0.31	0.41	2.50	0.76
1,1,1-Trichloro-3-nitro-2-propyl acetate	0.24	0.37	0.62	0.65
1,1-Diphenylhydrazine	0.68	0.35	4.67	1.94
1,4-Diphenylsemicarbazide	0.16	>4.40	>4.40	<0.04

[a] ED_{50} for stalk formation/ED_{50} for conidial formation.

hyphae and thus lacked selectivity in fungal response.

Effect of inhibitors of glycolate-glyoxylate metabolism.—The effect of inhibitors of glycolate oxidase and of two compounds that may affect glyoxylate metabolism is given in Table 2. All of the compounds were effective at sublethal concentrations. The most active were 2,4,6-tetrachloro and pentachlorophenoxyacetic acids, hexachloroisopropanol, and 1,4-diphenylsemicarbazide. The least effective was tetrachlorophenoxyacetic acid.

Effect of inhibition of macromolecular synthesis.—The data on purine analogues and other growth inhibitors are given in Table 3. Of the purine analogues, only 8-azaguanine and 2-thio-6-oxypurine inhibited formation at sublethal concentrations. Acridine and the two carbamates were effective, also.

Formation of spores.—The spore begins as a bud at the tip of the stalk and proceeds to enlarge. In the process it forms septa and pigment. Eventually, the conidium matures with longitudinal septa and a tail. Figure 2 illustrates the sequence starting when 2-day-old stalks are placed in darkness at 23 C.

Effect of gas mixture.—Low oxygen tensions had

TABLE 3. Toxicity of inhibitors of macromolecular synthesis to sporulation of *Alternaria solani*

Inhibitor	ED_{50} values (mM)			Toxic ratio[a]
	Stalk formation	Conidial formation	Stalk collapse	
8-Azaguanine	0.04	4.00	5.60	0.01
6-Chloropurine	>5.70	>5.70	>5.70	
2-Thio-6-oxypurine	1.61	6.00	6.00	0.27
2,8-Dithio-6-oxypurine	>5.00	>5.00	>5.00	
6-Thiopurine hydrate	5.88	>6.00	>6.00	
Acridine	0.17	0.67	>5.60	0.25
iso-Propyl-*N*-(3-phenyl)carbamate	0.12	0.31	>4.00	0.39
Ethyl-*N*-phenylcarbamate	0.52	0.91	6.18	0.57

[a] ED_{50} for stalk formation/ED_{50} for conidial formation.

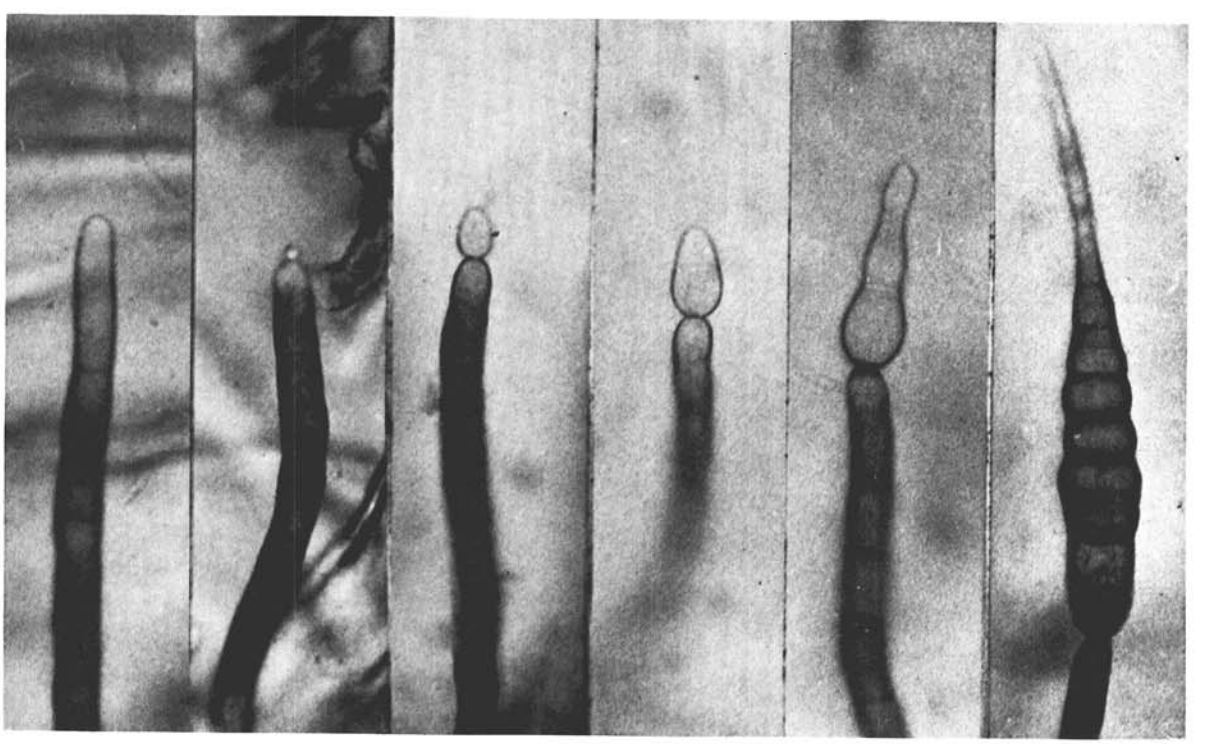

Fig. 2. The development of a conidium of *Alternaria solani*; (left to right) 2-day-old stalks at time of placing in darkness at 23 C for spore inducement; 3 hr of darkness, bud forms at tip of stalk; 5 hr of darkness, bud enlarges; 6 hr of darkness, septum forms; 7 hr of darkness, second septum forms; and 16 hr of darkness, spore matures.

little effect on spore formation. Percent sporulation of stalks at different oxygen tensions was 98, 83, 94, 96, 97 for oxygen concentrations of 20, 10, 5, 2.5, 1.25%, respectively. Even when the oxidation tubes containing stalks were flushed 5 times with nitrogen, sporulation was still 94%. Apparently residual oxygen following treatment suffices for spore formation. However, if stalks are grown under nitrogen and held in that atmosphere during spore induction, no spores are formed. Apparently the residual oxygen is exhausted at the time of spore induction and the process requires molecular oxygen.

Stalks grown in air and subjected to carbon dioxide treatments during spore induction sporulated at percentages of 98, 78, 74, 36, 8, and 0 for 0, 1, 3, 10, 20, and 50% enrichment with carbon dioxide, respectively. The ED_{50} value was 5.0% carbon dioxide, approximately the same as that for inhibiting formation of stalks. Spore formation was slightly more sensitive to carbon dioxide when it was mixed with nitrogen than to carbon dioxide mixed with air.

Effect of respiratory inhibitors.—Dosages of respiratory inhibitors required to inhibit spore formation 50% are presented in Table 1. Spore formation was highly sensitive to azide, whereas cyanide, fluoride, and iodide had little effect. All thiol reagents inhibited spore formation at concentrations that were not lethal to stalk elongation. Oligomycin displayed no effect against spore formation. Dinitrophenol as well as its 6-nonyl homologue were highly effective against spore formation. Undecanoic acid had a slight effect, but its unsaturated homologue, which is more fungitoxic than undecanoic acid, displayed little selective action. Dicoumarol and alloxan had no effect.

Effect of inhibitors of glycolate-glyoxylate metabolism.—The effects of these compounds are given in Table 2. With the exceptions of 4-nitrophenoxyacetic acid and 1,4-diphenylsemicarbazide, all of the compounds tested inhibited formation of spores at sublethal concentrations.

Effect of inhibition of macromolecular synthesis.—None of the purine analogues had any effect against spore formation (Table 3). However, acridine and the 2 *N*-phenylcarbamates were highly effective inhibitors of spore formation.

Differential action between stalk and spore formation.—In Tables 1-3, a column of toxic ratios is presented to distinguish effect on stalk formation from that on spore formation. Values less than 0.1 indicate selective action against stalk formation and values greater than 1.0 are arbitrarily designated to indicate selective action against spore formation. The range between 0.1 and 1.0 indicates little distinction between the two types of inhibition. The estimate of selectivity is conservative because the fungus had increased about 100-fold in cell number between the times of treatment in the two assays. Outstanding selective antistalk compounds were diethyldithiocarbamate, iodoacetic acid, oligomycin, 4-nitrophenoxyacetic acid, 1,4-diphenylsemicarbazide, and 8-azaguanine. Selective antispore compounds were azide, DNP, crotonic acid, 2,3,4,6-tetrachlorophenoxyacetic acid, and 1,1-diphenylhydrazine.

DISCUSSION.—In the genus *Alternaria*, asexual sporulation is a diversion from filamentous growth. The stalks arise from bud cells which form on the old hyphal cells (Fig. 1). The stalks grow as filaments in blue light and high temperatures. When a stalk is chilled or removed from blue light, it in turn produces a bud. The bud enlarges and eventually becomes a conidium. A portion of this sequence was first described partly by Rands (18) from the fungus in lesions on potato foliage.

It is not clear whether budding of hypha to form a filamentous stalk is at the expense of filamentous growth of more hyphae. However, on initiating a spore, a stalk stops growing as a filament. The metabolism of both processes appears similar. This metabolism of growth by budding may differ from that of filamentous growth, and these differences may be utilized to selectively inhibit budding.

Sporulation of most fungi is an aerobic process (5). *Alternaria tenuis* fails to sporulate when the oxygen tension falls below 1% (6). However, stalks of *A. solani* can sporulate in 12 hr in atmospheres of oxygen that approach zero. Stalks subjected to low oxygen tensions prior to spore induction fail to sporulate. Evidently, *A. solani* requires oxygen to sporulate, but the fungus very efficiently utilizes the last remnant of molecular oxygen under extremely low tensions. Apparently, the terminal respiratory system for formation of stalks and spores has a great affinity for oxygen.

Carbon dioxide is inhibitory to growth and development of *Alternaria*. ED_{50} values reported for inhibition of mycelial growth and spore germination of *A. tenuis* are about 20% (24). These values agree with those for inhibition of hyphal growth of *A. solani* in our studies. Values for inhibition of stalk and spore formation are much less (4.6 and 5.0%, respectively). Presumably, sporulation is 4-5 times more sensitive to carbon dioxide asphyxia than is growth. Wells & Uota (24) found that carbon dioxide stimulated growth of *A. tenuis* when oxygen was limiting. However, sporulation of *A. solani* was inhibited slightly more by carbon dioxide under lower than normal oxygen tensions.

The antisporulants listed in Tables 1-3 appear to act against the budding process. At effective concentrations, they do not inhibit mycelial growth in the stalk assay or elongation of stalks in the spore assay. Moreover, most of the antisporulants are about equally effective against stalk formation as against spore formation. Apparently, the affected metabolism is essential to the budding phase of both steps of sporulation, but probably not essential to filamentous growth. The antisporulants include inhibitors of respiration, thiol reagents, inhibitors of glycolate and glyoxylate metabolism, and inhibitors of synthesis of macromolecules that are required in cell division. The antisporulant response of *A. solani* to the metabolic inhibitors reveals in part the metabolism peculiar to sporulation.

The sporulation metabolism appears to terminate with falvin-dependent systems that contain iron. Sodium azide is the most effective inhibitor tested. Sodium cyanide and fluoride displayed little effect at concentrations permitting filamentous growth. Based upon the pKa value of hydrogen cyanide, its salt combines with hydrogen ions to form the undissociated form, a permeable species. Both azide and cyanide are potent inhibitors of iron- and copper-dependent systems, with cyanide being the more effective inhibitor (9). However, the lack of activity by cyanide suggests that metal containing enzymes of the cytochrome systems and polyphenol oxidases may not be involved in sporulation respiration. Because azide appears to permeate in the anionic form, the lack of activity of the fluoride ions is probably not due to poor permeation. Thus, none of the metal enzyme systems affected by fluoride appears essential for sporulation.

Toxicity of the thiol reagents to both steps in sporulation suggests that the budding process requires functional thiols. The less toxic response to spore formation may reflect a buildup of the thiol pool during the time between treatments in the two assays, or the greater distance the toxicant has to move to accumulate in the terminal cell of the stalk. Budding in the yeast, *Candida albicans*, involves thiol-dependent systems that are inhibited by cobaltous ion (17). The extreme sensitivity of stalk formation to diethyldithiocarbamate and iodoacetate suggests that these toxicants may act on critical sites lacking thiols or affect thiol systems that are not touched by the heavy metal or other thiol reagents.

Oligomycin is reported to inhibit oxidative phosphorylation at the step ADP to ATP (20). That oligomycin inhibits stalk formation indicates that the budding of hyphal fragments of *A. solani* to form stalks requires high energy compounds in the form of adenosine triphosphate (ATP). The lack of activity of oligomycin against spore formation may indicate that budding stalks may utilize other types of high energy phosphate; e.g., adenosine diphosphate (ADP), or that the fungus had built up a supply of ATP during the time between the treatments of the two assays.

Apparently, budding of both phases of sporulation requires greater concentrations of high energy phosphate compounds than mycelial growth because sporulation was the more sensitive to the uncoupling toxicants. Dinitrophenol (DNP) is known to abolish the synthesis of ATP coupled with mitochondrial respiration, to inhibit high energy exchange reactions, and to promote hydrolysis of ATP (20). Increasing hydrophobic bonding of DNP with alkyl substituents to the ring increases the uncoupling activity with mitochondrial preparations. Presumably, the increase in potency of the 6-nonyl homologue over that of DNP is due to increased capacity of the homologue for permeating cytoplasmic and mitochondrial membranes. The slight selective effect of the fatty acids may be due to uncoupling phosphorylation from respiration. The lack of activity by dicoumarol and alloxan may be due to their poor permeation to the site of action.

The budding stage of both phases of sporulation appears to involve the oxidation of glycolate to glyoxylate and its subsequent utilization. Previously, we reported (15) that certain phenoxyacetic acids and other compounds act by competing with glycolic acid for glycolate oxidase to inhibit spore formation in *A. solani*. Also, phenyl hydrazines were effective antisporulants (10). All of these compounds also effectively inhibited stalk formation. Hence, they are inhibitors of budding.

The compound, 4-nitrophenoxyacetic acid, inhibited stalk but not spore formation. The lack of activity toward spore formation was attributed to decomposition of the compound during the experiment (15). Apparently, the hyphal fragments in the stalk assay are highly sensitive to this compound, or perhaps they are inhibited by its decomposition products.

The phenylcarbazide, in which the *N*-substituted azo group prevents reactions with glyoxylate, lacks action against spore formation. Presumably the toxicity of the carbazide to stalk formation involves some mechanism not related to glyoxylate. This site may not be involved in spore formation, or the compound fails to migrate to that site at the terminus of the stalk.

Presumably, budding as well as other forms of growth involves the synthesis of nucleotides, nucleic acids, proteins, and other macromolecules. Through lethal synthesis, analogues of purines are incorporated into nucleotides and nucleic acids, and cause dysfunction of them. The two purine analogues toxic to stalk formation may act in this manner. The ineffective ones may fail to permeate to sites of nucleic acid synthesis. When stalks are treated with purines effective against stalk formation, the process of lethal synthesis may be too slow to be effective against spore formation. By the time stalks are formed, the fungus may have synthesized systems sufficient to grow for the course of the spore assay. Acridine acts directly on nucleic acids by being inserted between the base pairs (12). Nucleic acids with misalignments of base pairs fail to funtion. The direct action of acridine is indicated by its effectiveness against both stalk and spore formation.

The selective antisporulant action of the 2 *N*-phenylcarbamates presents an intriguing contrast between budding and filamentous growth. In higher plants, phenylcarbamates are known to inhibit cell division and in particular to interfere with normal mitosis (1). During budding of *Saccharomyces cerevisiae*, the nucleus divides directly by elongation, with one part being included in the bud cell to become the new nucleus of that cell (19). By contrast, in filamentous growth, cell division is incomplete. Nuclei divide some distance back from the hyphal tip, the center of high metabolic activity. Growth of the hypha occurs as a continual elongation of the terminal cell. Extension proceeds by an increase in protoplasm and synthesis of new cell wall. Thus, the switch from filamentous growth to budding on the initiation of stalk and spore of *A. solani* may proceed with nuclear division occurring in an

environment of high metabolic activity where the toxicant is likely to accumulate. Toxicants are known to permeate fungal cells of high metabolic activity more readily than dormant cells.

Although not conclusive, a study of fungal differentiation with inhibitors does indicate likely metabolic pathways peculiar to sporulation of *A. solani*. Verification may necessitate a study of the actions of inhibitors on affected systems in cell-free preparations. Because the action of antisporulants appears to be against budding, they may also inhibit other forms of fungal differentiation that involve a diversion from filamentous growth.

Inhibitors of budding may be useful as fungicides of high selectivity to control plant diseases. Fungicides of high selectivity are desirable to reduce hazards of their usage. Antisporulants have been shown to reduce plant diseases (8, 14). However, for practical control measures, effective means of application of antisporulants and their usage in conjunction with standard fungicides need to be developed.

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