The Effects of the Fungicide, Iprodione, on the Mycelium of *Sclerotinia sclerotiorum*

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ABSTRACT

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The growth of *Sclerotinia sclerotiorum* in solid and liquid medium was inhibited by 3 μM iprodione. At 24 μM, the growth of early log phase liquid cultures was inhibited completely within 4 hr. At the same concentration, however, iprodione had no effect on mycelial respiration 2 hr after exposure. Uptake of ^{14}C-labeled acetate, glucose, glucosamine, uracil, or thymine was affected only slightly by 60 μM iprodione. Incorporation of ^{14}C from glucose into nucleic acids was inhibited within 10 min by 3 μM iprodione. DNA incorporation of ^{14}C from thymine and uracil was inhibited 32%, whereas RNA incorporation of ^{14}C from uracil was inhibited only 15%. Purine and pyrimidine nucleotide incorporation of ^{14}C from aspartic acid and glycine and subsequent incorporation into RNA and DNA was not inhibited by 2.5 μM iprodione. It appeared unlikely that iprodione directly affected any major biosynthetic pathways or caused a major disruption of membrane permeability. The effect of iprodione on DNA synthesis appeared to be indirect.

Iprodione [3-(3,5-dichlorophenyl)-*N*-isopropyl-2,4-dioxoimidazolidine-1-carboxamide] is one of a group of new fungicides. It is particularly effective against *Sclerotinia* and *Botrytis* species (2). Iprodione is not phytotoxic to most higher plants and has a very low mammalian toxicity level. It has potential as an important protectant fungicide for a variety of vegetable and fruit crops (2).

The mechanism is not known by which iprodione and its analogues, procymidone [*N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-decarboximide, (7) and vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazoladine-2,4-dione] (1), affect susceptible fungi. The effects of these three fungicides on *Botrytis cinerea*, however, have been compared (9). All three fungicides inhibited mycelial growth much more than spore germination. The physiological processes of respiration, RNA synthesis, protein synthesis, and membrane permeability were not affected (9). The toxic effect of iprodione on *B. cinerea* appeared to be associated with DNA biosynthesis but also may have been due to disorders in

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lipid biosynthesis (4). Procymidone toxicity was suspected to have been caused by effects on membrane functions required for the transport process (7). Vinclozolin was reported to alter the triglyceride fraction of *Ustilago avenae* mycelia (1). In more recent studies, vinclozolin was thought to inhibit the cell wall synthesis of *B. cinerea* (3). The three fungicides caused a dose-dependent increase in mitotic instability in *Aspergillus nidulans* (5).

The following report has been prepared from studies on the mode of action on iprodione on *Sclerotinia sclerotiorum*, a potentially important pathogen of sunflowers. The intent of this study was to determine the specific site of action of iprodione in order to study the mechanism of resistance to the fungicide developed by some fungi.

MATERIALS AND METHODS

Fungus culture. *Whetzelinia sclerotiorum* (Libert) Korf et Dumont. (*Sclerotinia sclerotiorum*) strain 10940 was obtained from the American Type Culture Collection (ATCC). Cultures of *Sclerotinia* were maintained on potato-dextrose agar (PDA). Liquid shake cultures of *Sclerotinia* were grown in glucose peptone (GP) medium, 50 ml in 250-ml Erlenmeyer flasks. The GP medium consisted of glucose, 15 g; KH_2PO_4, 2.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; bacteriological peptone (from swine), 5 g; minor element concentrate A and B, 0.5 ml each; and distilled water to 1 L. Minor element concentrate A contained $ZnSO_4 \cdot 7H_2O$, 440 mg; $CuSO_4 \cdot 5H_2O$, 40 mg; $CaCl_2 \cdot 2H_2O$, 600 mg; NaCl, 260 mg; $(NH_4)_6 Mo_7 O_{24} \cdot 4H_2O$, 180 mg and distilled water, 100 ml. Minor element concentrate B contained $FeSO_4 \cdot 7H_2O$, 500 mg; $MnSO_4 \cdot H_2O$, 275 mg; and distilled water, 100 ml. The pH of the GP medium was adjusted to 6.4 with 1 N NaOH before autoclaving. All cultures were maintained at 22 C on a gyratory shaker.

Chemicals. Iprodione (Lot GD6, 630, 99.4% pure) and [^{14}C] iprodione, 23.8 mCi/mmol were gifts from the Rhône-Poulenc Company, Agrochimie 14-20, rue Pierre, Baizet, 69009 Lyon, France. The radiochemical substrates: [U-^{14}C]-glucose, 323 mCi/mmol; [U-^{14}C]glucosamine, 300 mCi/mmol; [2-^{14}C]acetate, 44 mCi/mmol; [2-^{14}C] uracil, 46.1 mCi/mmol; [2-^{14}C] thymine, 53.2 mCi/mmol; [U-^{14}C] glycine, 113 mCi/mmol and [U-^{14}C] aspartic acid, 225 mCi/mmol, were purchased from New England Nuclear, Boston, MA 02118. Other chemicals used in this investigation were reagent grade.

Growth studies. Growth curves for the fungus were determined as described by Reilly and Gottlieb (11). Iprodione was added to the growth medium in acetone solution. The initial concentration of acetone in treated and control cultures was 0.2%. The effect of various concentrations of fungicide on growth in solid medium was determined at 48 hr by measuring the diameter of colonies in petri dishes containing 10 ml of PDA. A 6-mm-diameter plug from an actively growing culture was used as an inoculum. The effect of the fungicide on the growth of liquid cultures was determined as described by Reilly and Gottlieb (11).

Fungal respiration. Mycelia were harvested from cultures after 36 hr of growth and suspended in GP medium (10 mg dry weight of mycelium per milliliter). Two milliliters of the suspension and 3 ml of GP medium were placed in the reaction chamber of a Yellow Springs Biological Oxygen Monitor, Model 59 (Yellow Springs Instrument Co., Yellow Springs, OH 45837) and oxygen consumption was determined with a Clark electrode. The procedure for extended periods of measurement was as described by Reilly and Gottlieb (10).

Uptake. Mycelia from 48-hr cultures were collected by vacuum filtration, washed with distilled water, suspended in fresh GP medium or 0.1 M potasium phosphate buffer (pH 6.5), and dispersed into 50-ml Erlenmeyer flasks (10 ml total, 3 mg dry weight per milliliter). Iprodione and ^{14}C substrate were then added simultaneously to the suspensions. The suspensions were maintained at room temperature on a reciprocal shaker at 150 strokes per minute (SPM). One-milliliter portions of the suspensions were removed at the times indicated to determine uptake. Mycelia were isolated by suction filtration on 10-μm Teflon Millipore filters (Millipore Corp., Bedford, MA 01730) and washed with 25 ml of distilled water. The mycelia and filters were placed in scintillation vials containing 3 ml of 5% trichloracetic acid (TCA). After 30 min, 10 ml of Insta-gel (Packard Instrument Co., Downers Grove, IL 60515) was added. Radioactivity for these and all other experiments was measured with a Packard Tri-Carb Model 3375 scintillation spectrometer.

Incorporation of ^{14}C-labeled substrates. Fungal suspensions were prepared as described in uptake experiments. Iprodione, and the ^{14}C labeled substrate, respectively, were added and the cultures incubated at room temperature on a reciprocal shaker 150 SPM. In the experiments with aspartic acid or glycine as the ^{14}C substrates, the cultures were not harvested. Instead, they were treated simultaneously with sublethal doses of iprodione and the ^{14}C-labeled substrate and then were incubated for 18 hr on a gyratory shaker. In all experiments, the mycelia were collected by suction filtration, washed with distilled water, and placed in centrifuge tubes with 5 ml of 5% TCA at 0–4 C. Cell fractions were isolated by the procedure of Gottlieb and Van Etten (6). Differential extraction of DNA and RNA was by the method of Schmidt and Thannhauser (12). The concentration of DNA and RNA was determined by the diphenylamine and orcinol methods (13), respectively.

Thin-layer chromatography (TLC). Glass plates (5 × 20 cm) coated with a 250-μM layer of silica gel HF were used for separations. Solvent systems used to separate cell wall constituents were: *n*-butanol:glacial acetic acid: water (60:30:10, v/v); *n*-propanol:ethyl acetate:water:25% ammonia solution (60:10:30:10, v/v). The R_f of authentic ^{14}C-labeled glucose and glucosamine were determined with a Packard Model 7201 radiochromatogram scanner. Major peaks were removed from experimental plates by scraping and ^{14}C was quantitated by liquid scintillation counting. Lipid extracts were fractionated by TLC with a hexane:diethyl ether: glacial acetic acid (70:30:2, v/v) solvent system. Nucleotides were chromatographed with 0.2 N HCl and detected with UV light. Major ^{14}C peaks were detected with the radiochromatogram scanner and quantitated as above.

RESULTS

Growth studies. Mycelial growth of *Sclerotinia sclerotiorum* was completely inhibited in solid and liquid medium by 3 μM iprodione. The ED_{50} was 0.6 and 0.9 μM in solid and liquid media, respectively (Fig. 1A and B). Complete inhibition of growth of early log phase mycelia in liquid medium was observed 4 hr after the addition of 24 μM iprodione (Fig. 2). After 6 hr, the dry weight of the mycelia began to decrease, possibly due to lysis.

Respiration. Oxygen consumption by the mycelia was inhibited only 2.5 and 5.8% by 3 and 300 μM, respectively. No inhibition of oxygen consumption was detected in early log phase mycelia treated for 2 hr with 24 μM iprodione. In contrast, 500 μM sodium azide caused a 73% reduction in oxygen consumption within 2 min.

Uptake. There was no effect on the uptake of ^{14}C glucose by the mycelia of *S. sclerotiorum* exposed to 60 μM iprodione over a 2-hr period (Fig. 3). Acetate and glucosamine uptake was reduced 9 and 23%, respectively, in 4 hr. Thymine uptake was reduced by 19.5 and 6% in separate 2-hr experiments and uracil uptake was stimulated by 10%.

Incorporation of ^{14}C substrates. The effects of iprodione on the incorporation of [U-^{14}C] glucose into various biochemical fractions was studied both as a function of time and as a function of iprodione concentration. Iprodione caused a 21% inhibition of ^{14}C incorporation into the nucleic acids within 10 min after treatment, the earliest analysis time (Fig. 4). This inhibition increased only slightly after 1 hr. The cell pool fraction was virtually unaffected during the first 30 min but it increased to 120% of the control by 1 hr. Some early effects on incorporation of ^{14}C into cell wall, protein, and lipid fractions were noted, but these effects had diminished within 1 hr. Incorporation of ^{14}C into nucleic acids was inhibited 32% by 3 μM iprodione, but inhibition increased to only 65% when the concentration of iprodione was increased to 300 μM (Fig. 5). Incorporation of ^{14}C into the cell wall fraction was affected similarly.

When ^{14}C glucose incorporation was measured 2 hr after

treatment with 30 μM iprodione, the nucleic acid and cell wall fractions were only 56.3 and 69.8% that of the control, respectively, and the cell pool fraction was 171.2% that of the control. These were clearly the most strongly affected fractions (Table 1). This effect was evident both when the data were compared with the control on DPM per milligram dry weight basis and when the data were expressed as a percent distribution of the total ^{14}C isolated. The major ^{14}C-labeled components of the cell wall hydrolysates were glucose and glucosamine. The ratio of glucose to glucosamine was the same in treated and control samples, and this ratio suggested that the reduction of ^{14}C incorporation into cell walls was a secondary effect of the fungicide.

Approximately 80% of the total ^{14}C was incorporated into the cell wall fraction when ^{14}C glucosamine was the substrate (Table 1). This incorporation was inhibited 20.5% by iprodione when the data were expressed on a DPM per milligram dry weight basis; when the data were expressed as percent of the total ^{14}C in each fraction, however, the cell wall fraction from treated mycelia was 104% that of the control. These data indicated that the increase in dry weight of cultures treated with the fungicide was reduced, but ^{14}C incorporation continued at the same rate as in the control cultures. Hydrolysates of cell wall fractions were analyzed by TLC. Glucosamine was the primary ^{14}C product in treated and control sample hydrolysates as determined by TLC analysis.

Sodium [2-^{14}C] acetate incorporation into various biochemical fractions was not greatly affected by 60 μM iprodione (Table 1).

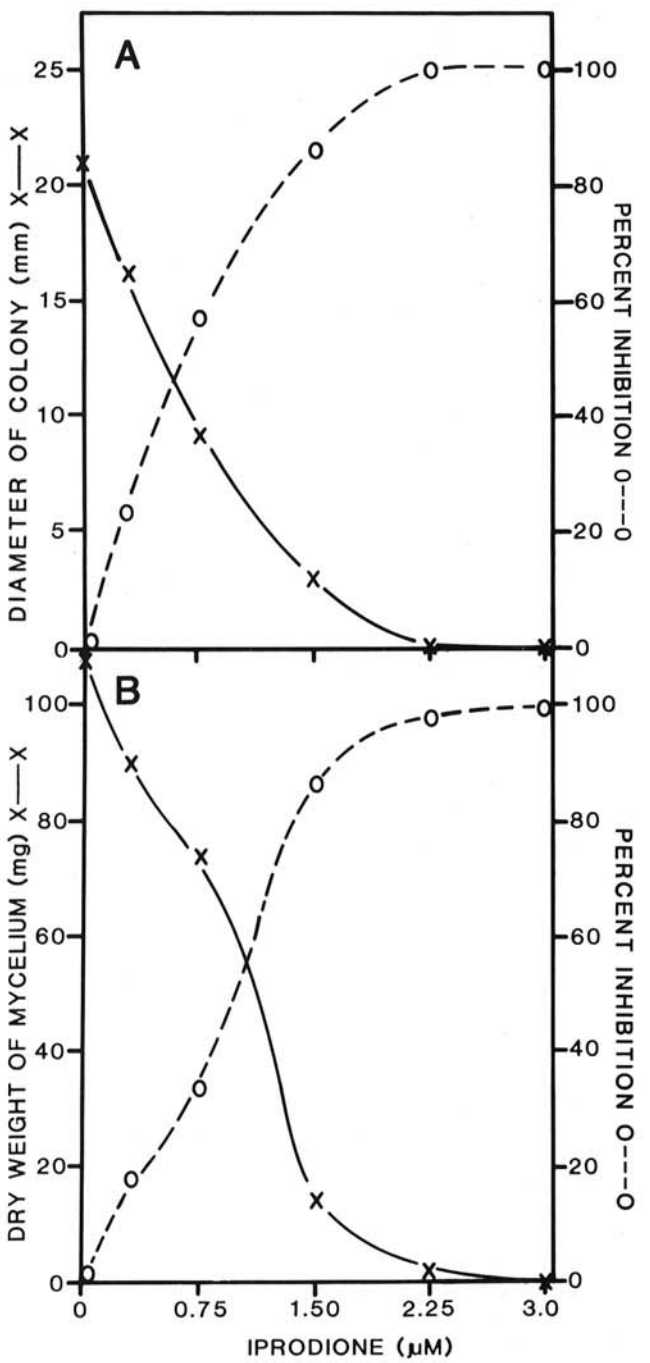

Fig. 1. Effect of iprodione concentration on growth of *Sclerotinia sclerotiorum* on solid and liquid medium. **A,** Colony growth on solid medium. **B,** Mycelial growth in liquid medium.

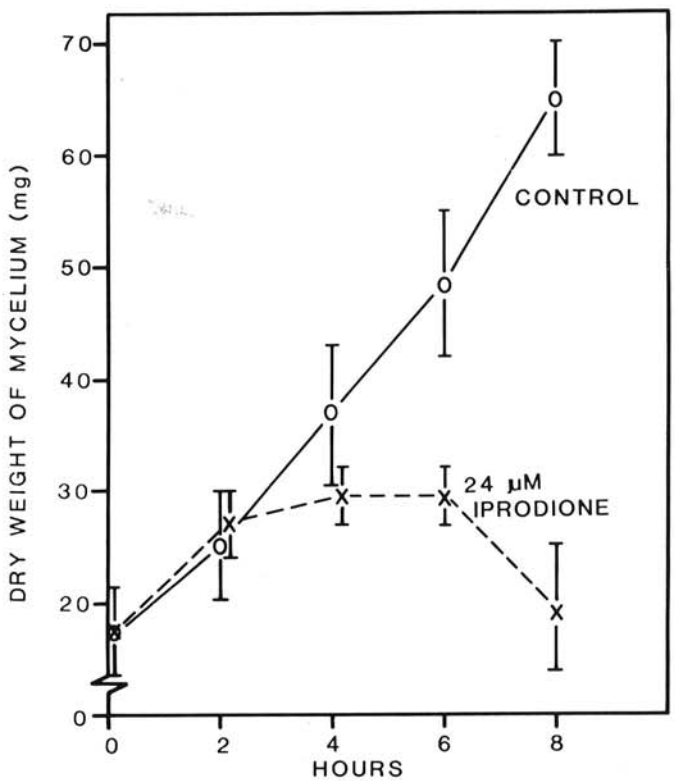

Fig. 2. Effect of 24 μM iprodione on growth of mycelia of *Sclerotinia sclerotiorum*. After 24-hr of growth, 24 μM iprodione was added to cultures and triplicate flasks were harvested at the times indicated to determine dry weights.

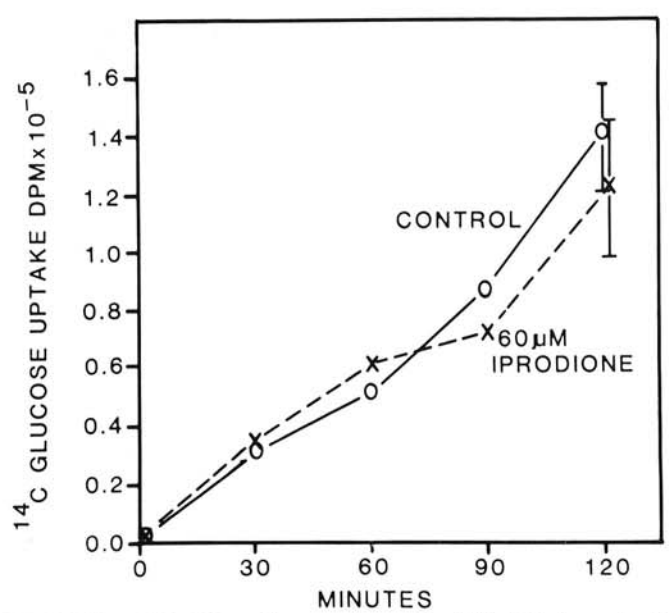

Fig. 3. Effect of 60 μM iprodione on the uptake of [U-^{14}C] glucose by the mycelia of *Sclerotinia sclerotiorum* as a function of time.

Lipids were the primary fraction to become ^{14}C-labeled and no difference was observed in the amount of ^{14}C in the lipid fraction from treated (54.1%) versus the control (53.9%). Thin-layer chromatography revealed no striking differences between lipid extracts from treated and control samples. Some reduction of ^{14}C incorporation into the protein fraction was noted, 11.5% in the treated vs 15.4% in the control; however, ^{14}C incorporation into the other fractions was very similar for the treated and control samples.

To further investigate the effects of iprodione on RNA and DNA synthesis, we used [2-^{14}C] uracil and [2-^{14}C] thymine as substrates. The RNA and DNA were selectively extracted from the mycelium. Incorporation of [2-^{14}C] uracil into RNA and DNA was

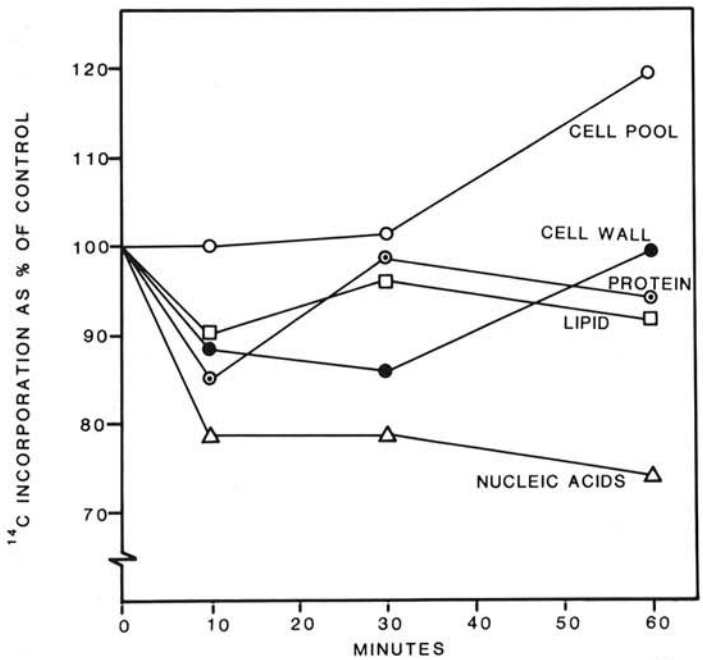

Fig. 4. The effects of 60 μM iprodione on the incorporation of [U-^{14}C] glucose into cell fractions of *Sclerotinia sclerotiorum* as a function of time. Cultures prepared in 0.1 M potassium phosphate buffer (pH 6.5) were treated with 60 μM iprodione and 5 μCi [U-^{14}C] glucose, then placed on a reciprocal shaker. The mycelia were harvested at the times indicated for cell fractionation.

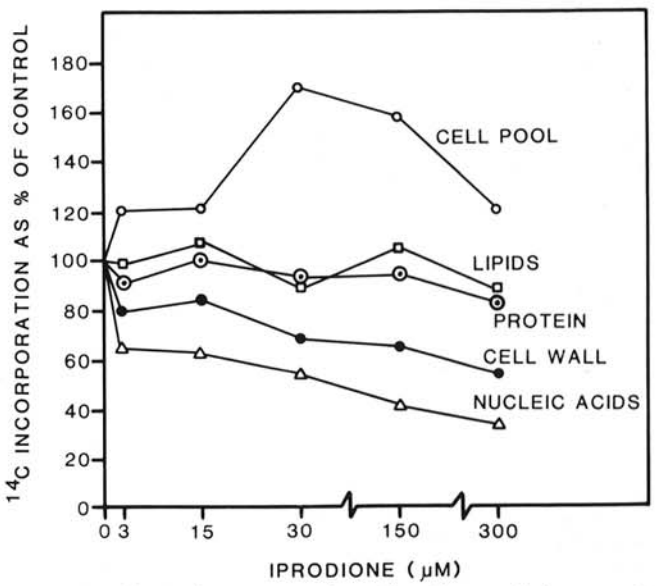

Fig. 5. Effect of increasing concentrations of iprodione on the incorporation of [U-^{14}C] glucose into cell fractions of *Sclerotinia sclerotiorum*. Cultures prepared in 0.1 M potassium phosphate buffer (pH 6.5) were treated with iprodione at the concentrations indicated and 5 μCi [U-^{14}C] glucose, then placed on a reciprocal shaker for 2 hr. The mycelia were then harvested for cell fractionation.

TABLE 1. The effects of iprodione on the incorporation of ^{14}C into cell fractions of *Sclerotinia sclerotiorum* with [U-^{14}C] glucose, [U-^{14}C] glucosamine, or sodium [2-^{14}C] acetate as the substrate

	Treatment[a]				
	Control		Iprodione		
Substrate cell fraction	DPM/mg dry weight[b]	Percent of radioactivity	DPM/mg dry weight	Percent of radioactivity	Percent of control
[U-^{14}C] Glucose[c]					
Cell pool	5,594	38.3	9,577	60.0	171.2
Lipids	738	5.0	664	4.2	90.0
Protein	1,898	13.0	1,702	10.7	90.0
Nucleic acids	3,199	21.9	1,800	11.3	56.3
Cell wall	3,172	21.7	2,215	13.9	69.8
Total radioactivity	14,601	99.9	15,958	100.1	109.3
[U-^{14}C] Glucosamine[d]					
Cell pool	6,249	14.3	4,081	12.2	65.3
Lipids	81	0.2	42	0.1	51.8
Protein	1,764	4.0	1,090	3.3	61.8
Nucleic acids	1,522	3.5	1,028	3.1	67.5
Cell wall	34,113	78.0	27,130	81.3	79.5
Total radioactivity	43,729	100.0	33,371	100.00	76.3
Sodium [2-^{14}C] acetate[e]					
Cell pool	56,355	20.0	65,456	22.8	116.1
Lipids	151,820	53.9	154,902	54.1	102.0
Protein	43,249	15.4	32,908	11.5	76.1
Nucleic acids	10,933	3.9	10,600	3.7	96.9
Cell wall	19,146	6.8	22,175	7.8	115.8
Total radioactivity	281,503	100.0	286,041	99.9	101.6

[a] All treatments were in triplicate with control cultures treated with acetone. The ^{14}C substrate and iprodione or acetone were added simultaneously.
[b] DPM=decomposition per minute per milligram dry weight of mycelium.
[c] Cultures were exposed for 2 hr to 5 μCi of [U-^{14}C] glucose and iprodione at 30 μM. The dry weight of mycelia was 41.0 $\pm$ 3.8 mg per treatment; potassium phosphate buffer (pH 6.5) was used to form the suspension.
[d] Cultures were exposed for 4 hr to 2 μCi of [U-^{14}C] glucosamine and iprodione at 30 μM. The dry weight of mycelia was 52.1 $\pm$ 3.2 mg per treatment; GP medium was used to form the suspension.
[e] Cultures were exposed for 4 hr to 10 μCi of sodium [2-^{14}C] acetate and iprodione at 60 μM. The dry weight of mycelia was 26.1 $\pm$ 2.7 mg per treatment; GP medium was used to form the suspension.

TABLE 2. The effect of iprodione on the incorporation of [2-^{14}C] uracil or [2-^{14}C] thymine into the RNA and DNA of *Sclerotinia sclerotiorum*

Substrate treatment[a]	Iprodione conc. (μM)	DPM RNA[b]	Percent of control	DPM DNA[b]	Percent of control
[2-^{14}C] uracil					
Control		9.76×10^5		1.18×10^4	
Iprodione	75	8.24×10^5	84.4	0.81×10^4	68.6
[2-^{14}C] thymine					
Control		9.87×10^4		9.49×10^2	
Iprodione	75	11.02×10^4	111.6	6.43×10^2	67.7

[a]Cultures suspended in GP medium were exposed to 75 μM iprodione for 30 min before the addition of the ^{14}C substrates. After an additional 3 hr, the cultures were harvested and incorporations of ^{14}C into RNA and DNA were determined.
[b]Differential extraction of RNA and DNA was conducted and total disintegrations per minute (DPM) per fraction was determined.

TABLE 3. The effect of iprodione on the incorporation of ^{14}C from [U-^{14}C] aspartic acid or [U-^{14}C] glycine into RNA and DNA of cultured *Sclerotinia sclerotiorum* mycelium

Substrate fraction sample	Iprodione conc. (μM)	Total DPM[a]	DPM/mg dry weight[b]	Percent of control
[U-^{14}C] Aspartic acid				
RNA				
Control		1.2×10^6	5,268	
Treated	2.4	1.2×10^6	10,075	191
DNA				
Control		3.5×10^5	1,536	
Treated	2.4	3.8×10^5	3,190	208
[U-^{14}C] Glycine				
RNA				
Control		4.5×10^6	19,754	
Treated	2.4	6.9×10^6	57,934	293
DNA				
Control		1.1×10^6	4,829	
Treated	2.4	1.8×10^6	15,113	313

[a]Cultures were exposed to 10 μCi of the ^{14}C substrates and at the end of the 18-hr period of growth approximately 90% of the ^{14}C was utilized by the mycelia in both treated and control cultures. DPM = disintegrations per minute.
[b]The cultures had 237.8 mg dry weight after 48-hr of growth. Iprodione, to give 2.4 μM, and the ^{14}C substrates were added and growth continued for an additional 18 hr. At the end of the 18 hr, the flasks contained an additional 227.8 or 119.1 mg dry weight for control or treated cultures, respectively. This was a 47.7% inhibition of growth.

suppressed 15.6 and 31.4%, respectively, by 75 μM iprodione. When [2-^{14}C] thymine was the precursor, RNA incorporation increased 11.6%, but DNA incorporation decreased 32% (Table 2).

Glycine and aspartic acid are precursors of purine and pyrimidines, respectively. The effect of iprodione on the incorporation of [^{14}C] glycine and [^{14}C] aspartic acid into purines and pyrimidines was determined indirectly by measuring ^{14}C incorporation into DNA and RNA. Incorporation of [^{14}C] aspartic acid into DNA and RNA was not affected by 2.4 μM iprodione over an 18-hr period when the analysis was based on total DPM in the respective fractions. When the analysis was based on the dry weight of cells, however, increased incorporation of ^{14}C into both DNA and RNA was noted. Although cell growth was inhibited 48%, RNA and DNA synthesis obviously was not inhibited under these assay conditions (Table 3).

Incorporation of ^{14}C from glycine into RNA and DNA also indicated that the synthesis of purines, pyrimidines, RNA, and DNA was not inhibited by sublethal doses of iprodione. The ^{14}C distribution pattern of glycine in RNA and DNA in the treated and control mycelia differed greatly. The treated mycelia had almost three times as much ^{14}C incorporation into both RNA and DNA as did the control mycelia (Table 3). A difference between the treated and control cultures in metabolic rates and utilization of substrates in major pathways may account for the different incorporation levels of glycine. The isolated RNA fraction of ^{14}C aspartic acid incorporation was hydrolyzed and analyzed for ^{14}C nucleotides by TLC (Table 4). Quantitative differences in ^{14}C incorporation existed between the control and iprodione-treated cultures,

TABLE 4. Distribution of ^{14}C in nucleotides separated by thin-layer chromatography (TLC) after RNA hydrolysis[a]

Nucleotide[b]	DNA Control	DPM Treated
2',3' AMP	469	610
2',3' GMP	322	416
2',3' CMP	253	166
2',3' UMP	188	80

[a]The RNA was extracted from the mycleium by hydrolysis in 1 N KOH at 37 C for 18 hr. Fifty microliters of this material, after neutralization, was developed on TLC plates in 0.1 N HCl. UV-fluorescent spots that appeared in the same area as cochromatographed authentic nucleotides were scraped and radioactivity was determined.
[b]AMP=adenosine monophosphate, GMP=guanosine monophosphate, CMP=cytidine monophosphate, and UMP=uridine monophosphate.

probably due to altered metabolism. It appeared that the biosynthetic pathways for purine and pyrimidine nucleotides were not directly inhibited by iprodione.

DISCUSSION

Inhibition of mycelial growth of *S. sclerotiorum* within 4 hr after the addition of iprodione to the cultures indicated that its action was rapid and that it probably affected a vital function of the cells. Iprodione, however, had no effect on respiration over extended periods, even at a concentration 20 times that required to inhibit growth. Uptake of ^{14}C-labeled substrates by mycelia exposed to iprodione varied only slightly from the controls, and this appeared to be a nonspecific response in that most substrates were slightly affected. We concluded that the fungicide did not directly affect energy production. Likewise, increased leakage of electrolytes from the cells due to iprodione was improbable. An early response with changes in membrane permeability is decreased respiration. For example, the polyenic antibiotic nystatin causes a rapid loss of potassium from fungal cells by altering membrane permeability. This loss of potassium stops respiration within 10 min (8). Our data were not consistent with observations associated with gross changes in cell permeability; therefore, this mode of action was not considered further.

We suspect that the decreased incorporation of ^{14}C glucosamine into the cell wall and slight reductions in protein synthesis were secondary effects of iprodione, owing to a decreased uptake of labeled substrates. In contrast, the nucleic acids were more strongly affected at lower concentrations and shorter exposure times (Figs. 4 and 5). The inhibition of ^{14}C incorporation into nucleic acids, however, was not as dramatic as would be expected if the polymerization of nucleotides was directly involved.

Our data indicated that ^{14}C incorporation into the DNA fraction was reduced twice as much as that into RNA when uracil or thymine were the substrates (Table 2). This inhibition, although probably not a primary effect, may be closely linked to the site of action of iprodione. In experiments in which cultures were treated with sublethal doses of the fungicide and allowed to incorporate aspartic acid or glycine into nucleotides, stimulation of ^{14}C incorporation was observed in the treated mycelia. These data indicated that iprodione did not directly act on biosynthetic processes related to DNA synthesis. Thus, inhibition of nucleotide

synthesis and nucleic acid synthesis were ruled out as possible modes of action of the fungicide.

Growth was the only vital process shown to be completely inhibited by iprodione. This inhibition could not be demonstrated until approximately 4 hr after treatment; however, an initial reduction of incorporation of ^{14}C substrates into nucleic acids could be demonstrated within 10 min. A possible explanation of these data is that nuclear division was in some manner disrupted by iprodione. Observations consistent with this explanation have been made with two structural analogues of iprodione. A 65% inhibition of growth and number of nuclei in the germ tubes of *Botrytis cinerea* exposed to procymidone for 2 hr was reported by Hisada et al (7). A 63% inhibition of cell multiplication and a 16% decrease in dry weight of sporidia of *Ustilago avenae* exposed to vinclozolin was reported by Buchenauer (1). Iprodione, procymidone, and vinclozolin induced mitotic instability in *Aspergillus nidulans* (5). The above observations with structural analogues of iprodione and our data appear to be consistent with the concept that the mode of action of iprodione is related to some interaction with the nucleus. Preliminary data from our laboratory indicates that iprodione selectively binds to proteins. The relationship of these observations to nuclear division is currently being investigated.

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