Effect of Pesticides on Growth and Sclerotial Production of Sclerotium rolfsii

G. A. Bozarth and B. G. Tweedy

Postdoctoral Fellow and Associate Professor, respectively, Department of Plant Pathology, University of Missouri, Columbia 65201.

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ABSTRACT

The effect of 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine (atrazine), 1,1-dimethyl-3-(*a,a,a*,-trifluoro-*m*-tolyl) urea (fluometuron), 3-(*p*-bromophenyl)-1-methoxy-1-methylurea (metobromuron), *a,a,a,*-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine (trifluralin), and thiram on mycelial growth and production of sclerotia by *Sclerotium rolfsii* was investigated. At 50 μg/ml, thiram completely inhibited mycelial growth and inhibition by the herbicides ranged from 26-75%, with fluometuron being the most inhibitory. All herbicides inhibited numbers of sclerotia produced, with fluometuron being the most inhibitory (83% at 50 μg/ml). Sclerotia produced on herbicide-treated medium were larger than those produced on control medium. The weights per sclerotium (expressed as per cent of control) of sclerotia produced on media containing 50 μg/ml pesticide were: fluometuron 223, atrazine 189, trifluralin 179, metobromuron 165, and thiram 85. Phytopathology 61:1140-1142.

Although several investigators have measured the effect of herbicides on the growth of *Sclerotium rolfsii* (1, 3, 4, 10, 11, 12, 13, 14), little is known about the effect of herbicides on the production of sclerotia (3, 5). Curl et al. (5) reported that sclerotium production was suppressed in soil cultures of *S. rolfsii* treated with 12 μg/g of 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine (atrazine). Bozarth (3) found that sclerotial initial formation was increased in soil treated with 1,1-dimethyl-3-(*a,a,a,*-trifluoro-*m*-tolyl)urea (fluometuron) at 1, 5, and 10 μg/g.

This study was initiated to evaluate the effect of several pesticides on growth and production of sclerotia by *S. rolfsii*. All pesticides used were of high purity (≧98%). Atrazine, fluometuron, *a,a,a,*-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine (trifluralin), 3-(*p*-bromophenyl)-1-methoxy-1-methylurea (metobromuron), and thiram were tested at concentrations of 0, 10, 50, and 100 μg/ml. All are herbicides except thiram. Potato-dextrose agar (Fisher Scientific Co., dehydrated) was distributed (300 ml/flask) prior to autoclaving. The sterile medium was cooled to 45-50 C, 5 ml of stock pesticide solutions in acetone was added to each flask, and the contents was thoroughly mixed by means of a magnetic stirrer. Agar media were dispensed in 10-ml portions into sterile disposable petri dishes (100 × 15 mm). The poured plates were placed on the laboratory bench for 24 hr prior to their inoculation with a 6-mm mycelial disc cut from the periphery of an actively growing colony of *S. rolfsii*. The culture of *S. rolfsii* was isolated from *Trifolium* sp. in Alabama. The six plates used for each treatment were incubated at 28 C. Radial growth determinations consisting of two diameter measurements were made at 24-hr intervals until the fungus in the control plates covered the surface of the agar. The data in Table 1 are based on the 2-day measurements. The plates were further incubated for 20-30 days when the sclerotia were collected by a suction apparatus, air-dried, and subsequently dried for 18 hr over anhydrous calcium sulfate. They were then weighed and counted. Each experiment was repeated at least once, with similar results being obtained in all cases.

All chemicals greatly inhibited growth at the 50- and 100-μg/ml treatments (Table 1). The four herbicides slightly inhibited radial growth of *S. rolfsii* at 10 μg/ml, and the fungicide, thiram, greatly retarded growth at this concentration. Fluometuron was the most effective of the herbicides in inhibiting radial growth, and atrazine was the least inhibitory. Growth was 25.8 and 75.1% of the controls at 50 μg/ml of these two compounds, respectively. At 50 μg/ml, metobromuron and trifluralin were intermediate in toxicity, but at 10 μg/ml, trifluralin was more inhibitory than the other herbicides. Although mycelial growth was greatly reduced in the thiram plates when measurements were made, the fungus in some plates later overcame this inhibition, covered the surface of the plates, and produced sclerotia of typical numbers and size.

The numbers of sclerotia produced decreased with increasing concentrations of herbicides (Table 2). However, in thiram containing plates where growth occurred, there was no appreciable effect on the number of sclerotia produced. All herbicides tested reduced sclerotial production at 10 μg/ml. At 50 μg/ml, fluometuron was most inhibitory to sclerotial production, reducing it by 83%, followed by metobromuron, atrazine, and trifluralin. Trifluralin reduced sclerotial production by 45% at 50 μg/ml.

The dry weight of sclerotia on herbicide-treated plates was usually inversely related to radial growth and sclerotial numbers. On plates treated with fluometuron, radial growth and sclerotial production were inhibited most, but the sclerotia were larger. The few sclerotia produced at higher concentrations of fluometuron were very large and irregular in shape. All herbicides at 50 μg/ml caused an increase in sclerotial

TABLE 1. Effect of selected pesticides on the radial growth of *Sclerotium rolfsii*[a]

Pesticide	Colony diam, % of control (μg/ml)			
	1	10	50	100
Atrazine	99.0[b]	95.8	75.1	58.0
Fluometuron	99.0	96.1	25.8	0.0
Metobromuron	101.2	95.9	70.4	34.4
Trifluralin	101.8	64.7	56.0	53.5
Thiram	72.6	27.6	1.3	1.8

[a] Medium was potato-dextrose agar (Fisher Scientific Co., dehydrated).
[b] Values represent an average of six replications.

size; those produced on media containing trifluralin, atrazine, or metobromuron were 1.7-1.9 times as large as on control medium, and those produced on fluometuron-treated medium were 2.2 times as large as the controls. Sclerotia produced on thiram plates were slightly smaller (0.9) than on control plates.

The four herbicides tested were all inhibitory to mycelial extension and sclerotial production. This study and other investigations (1, 3, 4, 11, 12, 13, 14) show that *S. rolfsii* is very sensitive to several herbicides. Although there are several reports in the literature where herbicides affected plant disease, few papers have dealt with the effect of herbicides on the disease caused by *S. rolfsii* (4, 7, 10). It has been reported (4, 7) that treatment of peanut fields with dinoseb resulted in increased yields. The increase in yield was attributed to a toxic effect of dinoseb on *S. rolfsii*. Peeples (10) found that field rates of S-ethyl dipropylthiocarbamate (EPTC) altered disease development by *S. rolfsii* in ladino clover and cotton in greenhouse studies.

These results suggest some interesting possibilities about what might happen in the field. Any compound that reduced the number of sclerotia produced would of course reduce the amount of inoculum overwintering, and therefore probably result in lower disease incidence.

The pattern of reduced mycelial growth, reduced sclerotial production, and increased size of sclerotia is difficult to explain based on information in the literature. Most workers agree that media influence the number and size of sclerotia produced. In many cases (2, 6, 8), sclerotial production was inversely related to mycelial development; however, the opposite effect was found with the herbicides used in this study. Milthorpe (9) observed two types of sclerotia in culture. The ones formed later in culture were usually larger than those produced earlier before mycelial growth stopped. He suggested that sclerotial size was influenced by staling products. This might explain why the sclerotia, although smaller in number, were larger in the herbicide plates. The herbicides may have inhibited some metabolic pathway, causing an accumulation of staling products (e.g., organic acids). Rodriguez-Kabana et al. (12, 13, 14) showed that herbicides can increase acid production by *S. rolfsii*. An increase in staling products thus appears to be the most logical explanation for the reduced numbers and increase in size of sclerotia observed on herbicide-treated media. Staling products may also account for the fewer and larger size of sclerotia produced on media high in nutrients, as under these conditions one would expect a greater abundance of staling products.

These studies suggest the need for further investigations to determine if herbicides alter the amount of disease caused by *S. rolfsii* in the field, the effect of herbicides on staling products, and the effect of staling products on the development of sclerotia.

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TABLE 2. Effect of selected pesticides on the production of sclerotia by *Sclerotium rolfsii*[a]

Pesticide	0 μg/ml		1 μg/ml		10 μg/ml		50 μg/ml		100 μg/ml	
	N[b]	W[c]	N	W	N	W	N	W	N	W
Atrazine	444[d]	0.261	395	0.291	314	0.333	172	0.493	100	0.694
Fluometuron	375	0.300	385	0.306	351	0.318	65	0.668	7	0.986
Metobromuron	460	0.258	447	0.267	310	0.349	105	0.427	70	0.292
Trifluralin	421	0.274	425	0.287	363	0.354	227	0.491	196	0.577
Thiram	404	0.315	373	0.340	363	0.310	410	0.269	380	0.265

[a] Medium was potato-dextrose agar (Fisher Scientific Co., dehydrated).
[b] N = Number of sclerotia produced per plate.
[c] W = Weight per sclerotium (mg).
[d] Values represent an average of six replications.

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