Effects of Herbicides on Carpogenic Germination of *Sclerotinia sclerotiorum*

V. L. RADKE, Former Graduate Research Assistant, and C. R. GRAU, Associate Professor, Department of Plant Pathology, University of Wisconsin, Madison 53706

ABSTRACT

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Of nine herbicides investigated, trifluralin, pendimethalin, metribuzin, simazine, and atrazine stimulated carpogenic germination of *S. sclerotiorum* as measured by the percentage of germinated sclerotia and by numbers of stipes and/or apothecia that developed per sclerotium. Linuron and DNBP inhibited germination and apothecium development, and alachlor caused variable responses. Simazine and atrazine enhanced stipe formation but stipes and apothecia were malformed. Metribuzin, also a triazine, enhanced stipe and apothecial growth without malformations.

Sclerotinia sclerotiorum (Lib.) de Bary is a soilborne plant pathogen with a wide host range, worldwide importance, and ability to survive in soil for long periods as sclerotia (19,26). Sclerotia germinate myceliogenically or carpogenically as described by Coley-Smith and Cooke (5). The latter type of germination results in apothecia that liberate ascospores, which serve as primary inoculum for diseases such as white mold of common bean (*Phaseolus vulgaris* L.) (1,2,23). Carpogenic germination of sclerotia is affected by such factors as depth of burial, soil moisture, soil temperature, light, transition metal compounds, and sugars (6,7,9,18,22,24).

Studies of the effects of soil-applied pesticides on carpogenic germination have been limited to fungicides and soil fumigants. For example, benomyl, dichlozine, dichloran, quintozene, thiophanate-methyl (10,24), and PCNB (16) inhibit stipe formation, but captan, thiophanate, and thiram are less inhibitory (10,24). Soil fumigants such as dazomet (12,14), metam-sodium, chloropicrin, and methyl bromide (15) prevent carpogenic germination and are fungicidal. However, dichloropropene and ethylene dibromide were reported to increase stipe formation (16). Mycelial growth and sclerotial initials of another sclerotium-forming fungus, *Sclerotium rolfsii*, were stimulated by dibromochloropropane at rates lower than 5.2 kg/ha (20).

Effects of soil-applied herbicides on carpogenic germination of *Sclerotinia sclerotiorum* were not reported in reviews by Katan and Eshel (13) and Altman and Campbell (3). Knowledge of stimulatory or inhibitory action by herbicides on carpogenic germination by *S. sclerotiorum* would contribute to basic knowledge of this destructive pathogen and could influence the development of strategies for its control.

Preliminary experiments with pendimethalin, trifluralin, chloramben, alachlor, and DNBP showed no significant effects on percentage of sclerotia that germinated, but pendimethalin and trifluralin increased the number of apothecia produced per sclerotium. Chloramben, alachlor, and DNBP suppressed apothecium production. Our preliminary results were the basis for the experimentation we report in this paper.

MATERIALS AND METHODS

Production and preconditioning of sclerotia. Autoclaved pieces of celery were seeded with ascospores of *S. sclerotiorum* and incubated at 20 C for 14 days. Sclerotia were separated from the

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celery tissue by running tap water and forceps. Sclerotia were air-dried for 1 wk and placed on moist filter paper in a petri dish, which was sealed with Parafilm and stored at 5 C for 30 days. These preconditioned sclerotia were used for experiments immediately or after further storage at 5 C.

Herbicides and application techniques. Herbicides used in the experiments were alachlor, chloramben, DNBP, linuron, metribuzin, pendimethalin, trifluralin, atrazine, and simazine. All herbicides, from commercial formulations, were solubilized in acetone.

For each treatment, 2,000 cm^3 of a peat/loam soil/sand mixture (1:1:2) was steamed for 30 min at 130 C and allowed to cool and aerate for 24 hr. Soil (732 g) was treated by atomizing 50 ml of an herbicide-acetone solution onto the soil surface and mixed thoroughly. Herbicides were added at rates of 0.5, 1.0 and 2.0 μg a.i./g of oven-dry soil mixture. Thirty-four grams of of soil mixture was placed in each of three or four replicate petri dishes 9 cm in diameter and allowed to aerate 12–18 hr before the addition of five to 10 sclerotia per dish. Untreated and acetone-treated soil mixtures were included in all experiments.

Incubation conditions and quantification of data. Sclerotia were incubated 30–41 days in a controlled-environment chamber under the following conditions: photoperiod of 16 hr at 22–33 $\mu E \cdot m^{-2} \cdot s^{-1}$, air temperature of 14–16 C, and soil saturated with sterile distilled water. The percentage of sclerotia that germinated and numbers of stipe initials and apothecia per sclerotium were recorded. Sclerotia were recorded as germinated if one stipe initial or apothecium was present at the end of the incubation period.

Statistical analysis. We examined treatments in an unbalanced two-way

Table 1. Effects of trifluralin, atrazine, simazine, metribuzin, and DNBP on germination and stipe and apothecium development by *Sclerotinia sclerotiorum*

Rates (μg/g)	Treatments[x] 1	2	3	4	5	6	7
				Final % germination (MSE = 2.6)			
0.0	...	...	...	...	...	65	90
0.5	0[y]	90	70	90	85	...	...
1.0	0	80	80	85	65	...	...
2.0	0	75	80	30	75	...	...
Mean	0	82	77	68	65	...	...
				No. of stipe initials after 41 days of incubation (MSE = 1.7)			
0.0	...	...	...	...	...	0.7	1.3
0.5	0.10	0.8	1.1	2.0	1.7	...	...
1.0	0.00	0.9	3.4	3.7	1.1	...	...
2.0	0.00	1.8	2.7	1.2	1.9	...	...
Mean	0.03	1.2	2.4	2.3	1.5	...	...
				No. of apothecia after 41 days of incubation (MSE = 1.6)			
0.0	...	...	...	...	...	1.9	2.9
0.5	0.0[z]	3.6	1.5	3.9	0.7	...	...
1.0	0.0	3.9	0.8	5.5	0.8	...	...
2.0	0.0	2.8	2.1	0.0	1.7	...	...
Mean	0.0	3.4	1.4	3.1	1.0	...	...

[x] Treatments: 1 = DNBP, 2 = trifluralin, 3 = simazine, 4 = metribuzin, 5 = atrazine, 6 = control-acetone, and 7 = control.

[y] Data are presented as the mean number of stipes or apothecia per sclerotium based on four replicates with five sclerotia per replicate.

[z] Numbers of apothecia do not distinguish between normal and abnormal caps.

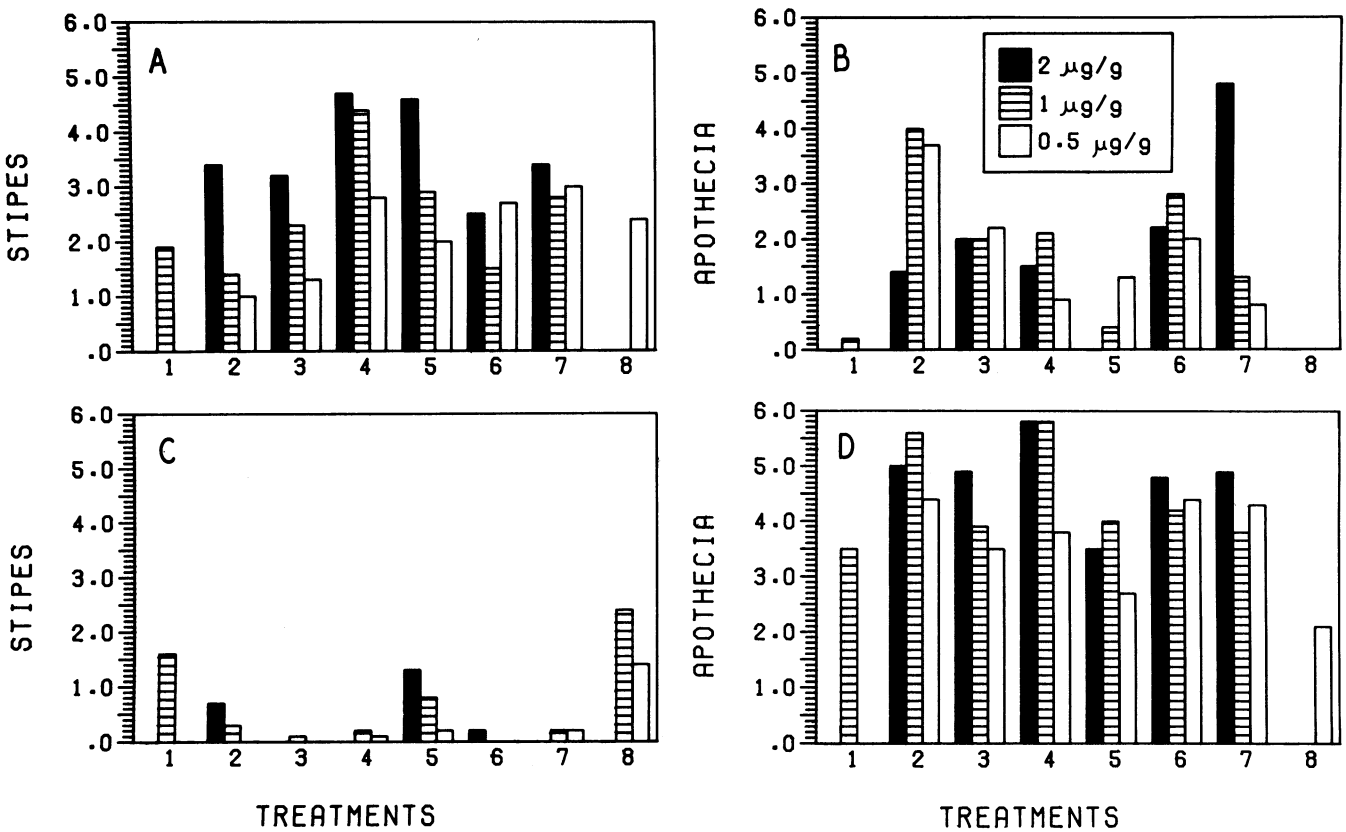

Fig. 1. Effect of herbicides on number of stipes and apothecia produced per sclerotium of *Sclerotinia sclerotiorum*. **(A and B)** Number of stipes and apothecia after 23 days of incubation, respectively; **(C and D)** number of stipes and apothecia after 37 days of incubation, respectively. Treatments: 1 = control (acetone), 2 = trifluralin, 3 = pendimethalin, 4 = metribuzin, 5 = alachlor, 6 = chloramben, 7 = linuron, and 8 = DNBP. Values are means of three replicates, eight sclerotia per replicate.

analysis of variance. The imbalance was due to having three each of two controls at rate 0 while all five treatments were at rates of 0.5, 1.0, and 2.0 μg/g. Mean separation was done with the Student-Newman-Keuls test on 17 factor levels. Analysis was repeated without DNBP to examine the effects of the remaining herbicide treatments and controls. To sort out treatment effects, additional analysis was done with and without controls. Transformations were used to stabilize the variance: logarithmic transformation for percentage of germination and square-root transformation for number of stipe initials and apothecia.

RESULTS

Experiment 1. A significant main effect of herbicides on percentage of germination was detected. This was due to suppression of carpogenic germination by DNBP. When the effect of DNBP was removed from analysis, a less significant effect of herbicides on percentage of germination remained. Significant differences between treatment means and the means of the controls for percentage of germination and numbers of apothecia per sclerotium occurred late in the experiments.

Early in this experiment, percentage of germination was enhanced, although not significantly over controls, by the following treatment-rate combinations: metribuzin at 0.5 and 1.0, simazine at 1.0 and 2.0, trifluralin at 2.0, and atrazine at 0.5 μg/g (Table 1). Reduced germination was exhibited by sclerotia treated with DNBP at all rates, atrazine at 1.0 and 2.0, simazine at 0.5, and trifluralin at 0.5 μg/g. By 41 days, germination was significantly reduced by DNBP at all rates and by metribuzin at 2.0 μg/g. All other treatment-rate combinations did not differ significantly from controls (Table 1 shows data at day 41 of incubation only). The number of stipe initials was statistically greater for sclerotia treated with simazine at 1.0 and 2.0, metribuzin at 0.5 and 1.0, atrazine at 0.5, and trifluralin at 2.0 μg/g than for the controls after 27 days of incubation.

Reduced numbers of stipe initials occurred with atrazine at 1.0, trifluralin at 0.5, and metribuzin at 2.0 μg/g, but only DNBP caused a statistically significant reduction. By day 41 of incubation, significant reduction in numbers of stipe initials had occurred for DNBP at all rates and for trifluralin at the high rate (2.0 μg/g) (Table 1). No apothecia had formed for any treatment after 27 days of incubation. By day 41, however, significant differences were apparent for the number of apothecia developed. Trifluralin and metribuzin had stimulating effects but DNBP was inhibitory. Effects of simazine and atrazine were not different from the control or from each other (Table 1). The triazine herbicides, metribuzin, atrazine, and simazine, differentially affected apothecium formation. Sclerotia incubated in metribuzin-treated soil produced larger apothecia than sclerotia incubated in acetone-treated soil (Fig. 1). Most stipe initials did not develop into mature, functional apothecia during 41 days of incubation when soils were treated with simazine and atrazine (Table 1). In addition, stipe initials were altered in morphology by atrazine and simazine; their tips darkened prematurely and became necrotic. If apothecia developed, discs were reduced in size and developed a "fluted" edge or were otherwise malformed (Fig. 2). In most cases, the apothecial disc did not properly expand.

Experiment 2. To encompass a wider range of herbicides, experiments containing the following herbicides were performed. (Of four trials, the data presented are for the final trial only.) The herbicide groups were phenolic (DNBP), dinitroaniline (trifluralin and pendimethalin), benzoic (chloramben), urea (linuron), amide (alachlor), and triazine (metribuzin). Sclerotial germination ranged from 0 to 89% with DNBP in all experiments. With all other herbicides, germination was 84% or greater after 41 days of incubation. Except for DNBP, final percentages of germination did not differ significantly from the controls.

Trifluralin consistently enhanced the number of apothecia produced per sclerotium as did metribuzin at 1.0 and 2.0 μg/g. Pendimethalin enhanced the number of apothecia per sclerotium but less consistently than trifluralin. DNBP delayed apothecium formation at 0.5 μg/g and completely inhibited it at 1.0 and 2.0 μg/g (Fig. 3). Alachlor and chloramben had variable effects on stipe and apothecium development. Initially, linuron, at high concentrations, induced formation of more apothecia than with acetone-treated sclerotia (Fig. 3). Formation of stipe initials was enhanced by trifluralin (1.0 and 2.0 μg/g), pendimethalin (all rates), chloramben (all rates), and linuron (2.0 μg/g) in the early part of the incubation period (Fig. 3); stipe initials continued to mature, which resulted in greater numbers of apothecia than with acetone-treated sclerotia (Fig. 3). All herbicide-treated sclerotia except those treated with DNBP deteriorated faster than untreated sclerotia.

Experiment 3. To determine whether durations of exposure to herbicides affected apothecium formation, preconditioned sclerotia were incubated on herbicide-treated soil for 7, 14, or 28 days before transfer to untreated soil and further incubation for 35 days. Germination, number of stipe initials, and number of apothecia that formed for each exposure period were compared with corresponding data for sclerotia continuously incubated in untreated soil for 35 days. Only the 2.0-μg/g rate of herbicide was used. Germination was not significantly altered as the length of exposure time to herbicides was increased except with DNBP. Germination of sclerotia treated with DNBP was reduced as exposure time increased. Sclerotia exposed to DNBP for 28 days or longer did not germinate.

Formation of apothecia was significantly reduced for sclerotia exposed to DNBP at 7, 14, and 28 days compared with the untreated control. Sclerotia incubated in soil treated with trifluralin formed more apothecia than did sclerotia incubated in untreated soil. Exposure for 28 days induced significantly more apothecia than developed in the control. The remaining herbicides did not significantly affect apothecium production as exposure time increased. Herbicides were apparently taken up rapidly by the sclerotia within the first 7 days of incubation (Fig. 4).

DISCUSSION

All herbicides that we evaluated affected some stage of the germination

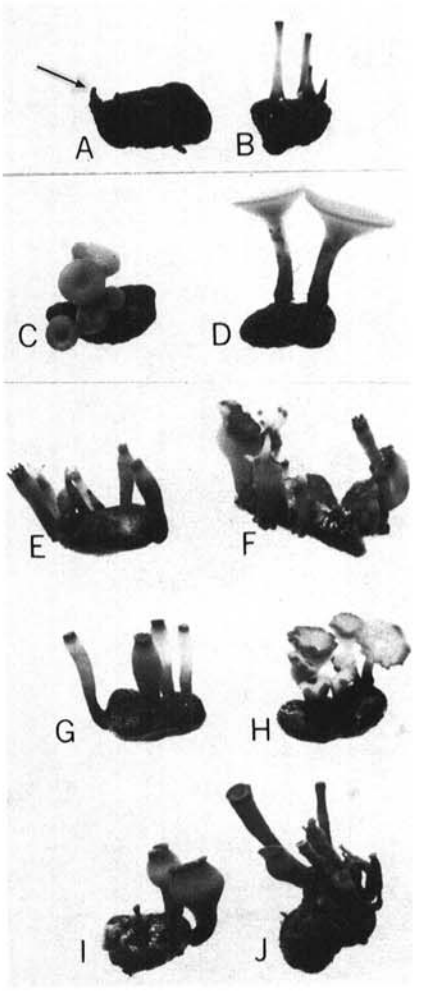

Fig. 2. (A–D) Sclerotia of *Sclerotinia sclerotiorum* showing normal development of stipe initials (arrow), stipes, and mature apothecia. **(E–J)** Sclerotia of *S. sclerotiorum* showing the identical effects of atrazine and simazine on development of stipes and apothecia.

process of *S. sclerotiorum*. Phenolic (DNBP), dinitroaniline (trifluralin and pendemethalin), and triazine (atrazine, metribuzin, and simazine) herbicides influenced the germination process more than benzoic (chloramben) and amide (alachlor) herbicides.

Phenolic herbicides like DNBP destroy membranes and prevent ATP formation in higher plants (4). We speculate that DNBP showed a similar mode of action against *S. sclerotiorum*. This herbicide completely inhibited germination at higher concentrations and greatly suppressed germination and stipe and apothecium formation at lower concentrations. Less than a 7-day exposure period was needed to affect carpogenic germination.

Dinitroaniline herbicides inhibit root and shoot growth through interference with cell division (4). Besides herbicidal activity, dinitroaniline herbicides also inhibit growth and reproduction of several plant-pathogenic fungi (8,21, 22,25), and they inhibit mycelogenic germination of sclerotia formed by *S. rolfsii* (22). In our studies, however, trifluralin and pendemethalin stimulated the formation of stipe initials and apothecium formation by *S. sclerotiorum*. Why a compound believed to be a mitotic inhibitor would enhance processes that involve cell division and elongation is unknown. Alachlor, an amide herbicide, also enhanced stipe and apothecium formation but to a lesser degree than the dinitroaniline herbicides. Amide herbicides are also reported to inhibit growth of higher plants by inhibiting root elongation through interference with cell division (4).

Triazine and urea herbicides are reported to function as inhibitors of photosynthesis (4). In our studies, triazine herbicides stimulated stipe and/or apothecium formation by *S. sclerotiorum*. All three triazines enhanced formation of stipe initials, but only the asymmetrical triazine herbicide, metribuzin, enhanced normal apothecium formation. The symmetrical triazines, atrazine and simazine, did not enhance apothecium formation but in fact caused abnormal stipe and apothecium development. The formation of stipe initials is not light-dependent, but normal maturation of apothecial caps is highly light-dependent (18). Presumably, triazines influenced both light-dependent

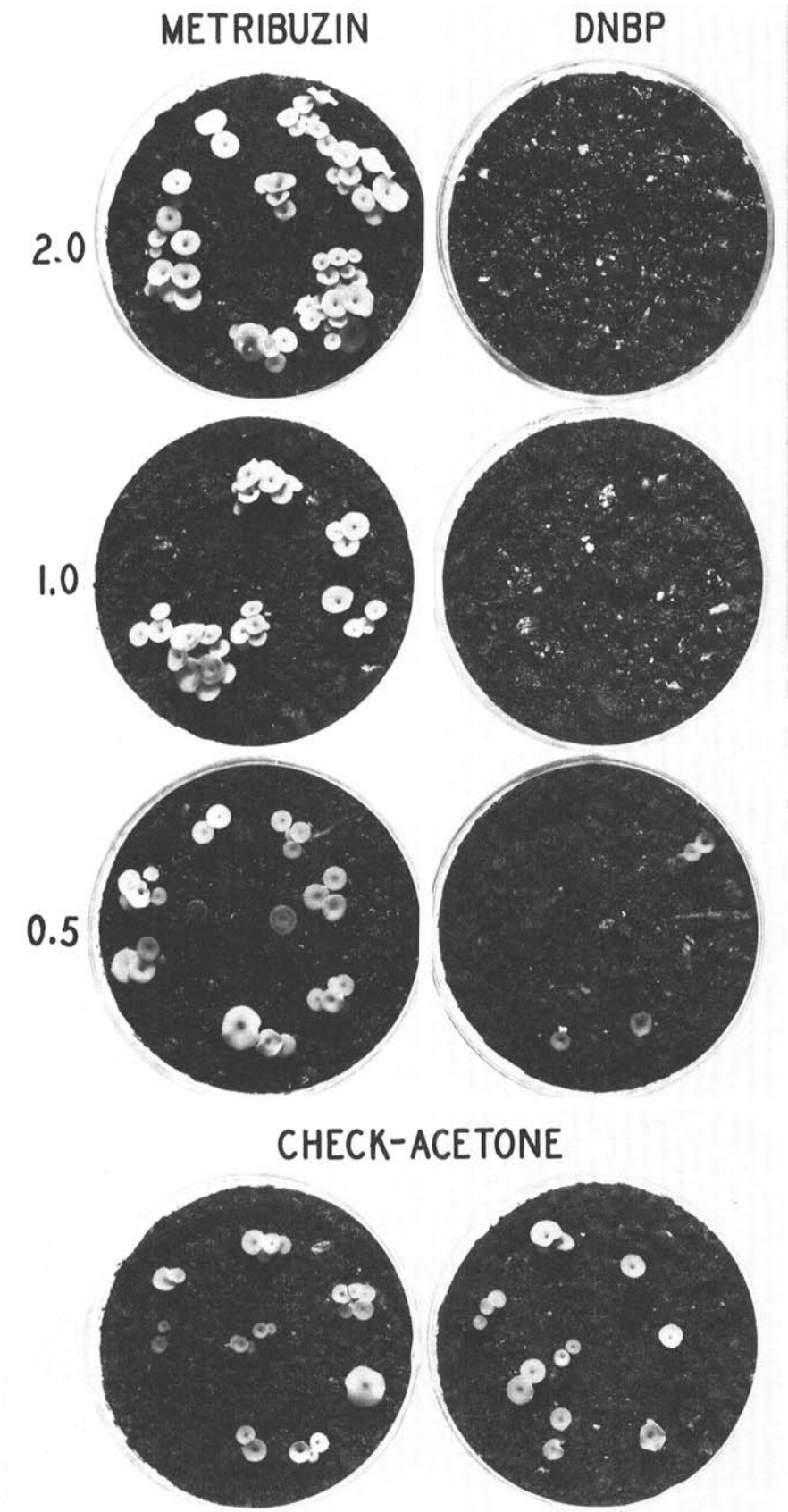

Fig. 3. Effect of metribuzin and DNBP (0.5, 1.0, and 2.0 μg/g of oven-dried soil) on sclerotial germination and stipe and apothecium formation by *Sclerotinia sclerotiorum*.

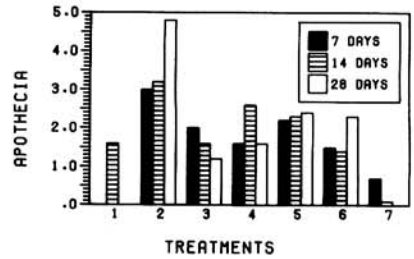

Fig. 4. Effects of herbicides on number of apothecia produced per sclerotium of *Sclerotinia sclerotiorum* when exposed for 7, 14, and 28 days. Treatments: 1 = control (acetone), 2 = trifluralin, 3 = metribuzin, 4 = linuron, 5 = chloramben, 6 = alachlor, and 7 = DNBP. All herbicides were applied at a rate of 2.0 μg/g of oven-dried soil. Values are means of three replicates, five sclerotia per replicate.

and independent germination processes of *S. sclerotiorum* because both stipe formation and cap maturation were affected. Atrazine is also reported to enhance the growth of *Fusarium solani* f. sp. *pisi* in soil (17). More propagules of *F. solani* f. sp. *pisi* were recovered from atrazine-amended soil than from untreated soil. Microconidial germination was shown to be stimulated by atrazine (17) and may explain the recovery of more propagules of *F. solani* f. sp. *pisi*. The severity of pea root rot caused by *F. solani* f. sp. *pisi* was greater in atrazine-amended than in unamended soils. More research is needed on the effects of triazine herbicides on the activities of soilborne pathogens.

A 7-day exposure to selected herbicides was enough time to influence carpogenic germination of *S. sclerotiorum*. Jacques and Harvey (11) reported that trifluralin concentrations of 0.25 μg/g still persisted 50–75 days after application. Thus it is conceivable that soil concentrations of trifluralin or other herbicides would be adequate long enough into the growing season to alter carpogenic germination of *S. sclerotiorum* in the field. Specific herbicides were shown to affect carpogenic germination, but it is not known if such herbicides can alter the germinative responses of sclerotia to governing factors such as moisture and temperature. Specific herbicides, by altering responses, could alter the accuracy of predictors used in disease forecasting models for this pathogen. The effects of herbicides on carpogenic germination by *S. sclerotiorum* should be studied in natural agro-ecosystems.

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