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ORIGINAL ARTICLE

Herbicides affect the carpogenic development and management of *Sclerotinia sclerotiorum*

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ABSTRACT

Paduan, F.N.; Osipe, P.B.; Sauer, A.V.; Canteri, M.G. **Herbicides affect the carpogenic development and management of *Sclerotinia sclerotiorum*. *Summa Phytopathologica*,** v.50, p.1-6, 2024.

Sclerotia of *Sclerotinia sclerotiorum*, a fungal pathogen of soybean, may be affected by herbicides used in the pre-planting management or even in post-emergence applications. The objective of the present study was to verify the action of the herbicides glyphosate, glufosinate ammonium and dicamba on mycelial growth and carpogenic germination of sclerotia and *Sclerotinia sclerotiorum*. The PDA culture medium was supplemented with concentrations of 10, 100, 1000 and 10000 ppm herbicides, receiving inoculation of fungal mycelium in Petri dishes. The diameter of the mycelium growth was measured daily. The carpogenic germination of sclerotia was analyzed with the deposition of ten sclerotia on the ground surface which was sterilized, arranged in plastic boxes and sprayed with solutions at concentrations of 1, 10, 100, 1000 and 10000 ppm herbicides. Evaluations were carried out at 30, 40 and 50 days after incubation by counting the number of stipes and apothecia. Glufosinate ammonium caused the greatest mycelial growth inhibition on *S. sclerotiorum*, which was above 40% even at the lowest concentration. The speed of mycelial growth was also lower from 10 ppm glufosinate. While for the control sample there was differentiation of stipes into apothecia, sclerotia treated with the herbicides showed a high number of stipes and a low number of apothecia. Changes in morphology, such as deformation on the edges of the apothecia disks, changes in coloration and stunted aspect, were also observed in sclerotia treated with herbicides. The herbicides glyphosate, glufosinate ammonium and dicamba alter the development of *S. sclerotiorum* sclerotia and may have an inhibitory action on the fungus.

Keywords: sclerotia, glyphosate, glufosinate ammonium, dicamba

RESUMO

Paduan, F.N.; Osipe, P.B.; Sauer, A.V.; Canteri, M.G. **Herbicidas afetam o desenvolvimento carpogênico e o manejo de *Sclerotinia sclerotiorum* *Summa Phytopathologica*,** v.50, p.1-6, 2024.

Escleródios do fungo patogênico da soja *Sclerotinia sclerotiorum* podem sofrer ação de herbicidas utilizados no manejo de aplicações pré-plantio ou mesmo pós-emergenciais. O objetivo foi verificar a ação dos herbicidas glifosato, glufosinato de amônio e dicamba no crescimento micelial e na germinação carpogênica dos fungos escleródios e *Sclerotinia sclerotiorum*. O meio de cultura BDA foi suplementado com concentrações de 10, 100, 1.000 e 10.000 ppm de herbicidas, recebendo inoculação de micélio fúngico em placas de Petri. O diâmetro do crescimento do micélio foi medido diariamente. A germinação carpogênica dos escleródios foi analisada com a deposição de dez escleródios na superfície do solo que foi esterilizado, disposto em caixas plásticas e pulverizado com soluções nas concentrações de 1, 10, 100, 1000 e 10000 ppm de herbicidas. As avaliações foram realizadas aos 30, 40 e 50 dias após a incubação por meio da contagem do número de estipes e apotécios. O herbicida glufosinato de amônio causou a maior inibição do crescimento micelial em *S. sclerotiorum*, com inibição acima de 40% mesmo na menor concentração. A velocidade de crescimento micelial também foi menor a partir de 10 ppm de glufosinato. Enquanto que para a amostra controle houve diferenciação de estipes em apotécios, os escleródios tratados com os herbicidas apresentaram elevado número de estipes e baixo número de apotécios. Alterações na morfologia, como deformação nas bordas dos discos dos apotécios, alterações na coloração e aspecto atrofiado também foram observadas nos escleródios tratados com herbicidas. Os herbicidas glifosato, glufosinato de amônio e dicamba alteram o desenvolvimento dos escleródios de *S. sclerotiorum* e podem ter ação inibitória sobre o fungo.

Palavras-chave: escleródios, glifosato, glufosinato de amônio, dicamba

Conventional soybean cultivation systems use chemical control of weeds. Herbicides can be applied before planting or even after establishment of the crop, which is possible due to the technology of resistant transgenic cultivars. Glufosinate ammonium, a non-selective herbicide of broad-spectrum action, has been increasingly used in the last decade, especially as an alternative after the ban on paraquat herbicide in Brazil (1, 15). However, glyphosate is still the most widely used herbicide worldwide (5). Glufosinate ammonium, glyphosate and dicamba are some of the herbicides used in genetically modified and resistant soybean cultivars.

A biotic factor that also affects the soybean crop is the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, the causal agent of white mold; it is capable of reducing the crop productivity by 20% to 30% (13). An important feature of this microorganism is its ability to produce sclerotia, survival structures that allow it to remain in the soil for long periods, serving as a source of primary inoculum (17).

The toxicity of herbicides is well known to affect biodiversity and therefore should also be investigated in microorganisms that cause plant diseases. Herbicides used in pre-planting desiccation management and even after crop emergence can extend their effects on phytopathogens of the soybean plant. There are reports of pre-emergence herbicides, such as butralin and pendimethalin, that affected the occurrence of white mold on bean plants and sclerotium germination on *S. sclerotiorum* (10). Vrisman et al. (16) also observed that sclerotium germination was negatively impacted by herbicides such as lactofen, sethoxydim, imazaquin, trifluralin and metribuzin. Lehner et al. (12) also mentioned that the herbicide S-metolachlor has a potential use in the management of *S. sclerotiorum* in beans. The present study investigated the action of glyphosate, glufosinate ammonium and dicamba on the mycelial growth and carpogenic germination of *S. sclerotiorum* sclerotia.

MATERIAL AND METHODS

Mycelial growth of *Sclerotinia sclerotiorum* subjected to herbicides

The fungus *Sclerotinia sclerotiorum* was subjected to concentrations of 1, 10, 100, 1000 and 10,000 ppm of the herbicides glyphosate (Glyphotal, 480 g. L^{-1} a.i.); glufosinate ammonium (Fascinate, 200 g. L^{-1} a.i.) and dicamba (Dicamax, 480 g. L^{-1} a.i.). The different concentrations were obtained by dilution and added on Potato Dextrose Agar (PDA-Sigma-Aldrich) culture medium.

After autoclaving, an Erlenmeyer flask containing PDA was cooled with the addition of the stock solution to the products, so that the desired final concentrations were obtained. The culture medium containing the different concentrations of the products were poured into Petri dishes, while the control consisted of PDA culture medium alone. After the medium had solidified, the plates received 6mm-diameter mycelial disks taken from the edges of the fungus grown for 2 days. The plates were incubated at 25 ± 2ºC and 12-hour photoperiod.

Mycelial growth was assessed daily, through measurements in two perpendicular directions, until the control treatment reached the edges of the plate, according to a methodology adapted from Avozani et al. (2). Growth Inhibition (GI) was determined using the formula:

$$GI = 100 - \frac{(i \times 100)}{t}$$

Where i corresponds to the radius of mycelial growth at the tested concentrations and t is the radius of the mycelial growth of the control.

The Mycelial Growth Speed Index (MGSI) was calculated by the formula:

$$MGSI = \sum \frac{(D - Da)}{N}$$

Where D corresponds to the actual average diameter of the colony, Da is the average diameter of the previous day's colony, and N is the number of days after inoculation.

The experiment was carried out twice. Experimental design was completely randomized, containing 5 treatments per herbicide, and 5 replicates. The mycelial growth inhibition data and the growth speed index were checked for normality according to Shapiro-Wilk test and homogeneity based on Bartlett's test. The data were then subjected to analysis of variance and Scott-Knott test at 5% probability.

Carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* subjected to herbicides

Artificially produced sclerotia were used on grain substrates of oat husk. For the substrate, 400 g hulled oats and 60 mL distilled water placed in Erlenmeyer flasks were used. They were autoclaved for 20 minutes at 120°C, added of 40 mL water and autoclaved again.

After cooling in a laminar flow chamber, 40 PDA disks containing mycelium of the fungus were transferred to Erlenmeyer flasks and incubated at 28 ± 2ºC for 30 days. The sclerotia formed on the oat husk substrate were dried at room temperature and manually separated from the substrate of origin (6).

The soil was autoclaved twice, 24 hours apart, at a temperature of 120°C for 30 minutes. After 24 hours in a drying oven, the soil was distributed at the rate of 150 grams per polystyrene box (11 cm x 11 cm) and ten sclerotia were placed under the surface of the soil (14).

Simulating a water depth of 3.5 mm, solutions were sprayed with concentrations of 1, 10, 100, 1000 and 10000 ppm of the herbicides glufosinate ammonium (Fascinate, 200 g. L^{-1} a.i.), glyphosate (Glyphotal, 480 g. L^{-1} a.i.) and dicamba (Dicamax, 480 g. L^{-1} a.i.) and concentrations of 0.1, 1, 10 and 100 ppm of the fungicide dimoxystrobin (Spot® SC, 200 g. L^{-1} a.i.). The plastic boxes were incubated at 19°C and 12-hour photoperiod. Evaluations were carried out at 30, 40 and 50 days by counting the number of stipes (NS) and the number of apothecia (NA) (14).

A completely randomized design with four replicates was adopted. Each box corresponded to a replicate. Data were transformed into $\sqrt{x}$ as it did not show a normal distribution according to Shapiro-Wilk test. After evaluation of the assumptions, the data underwent analysis of variance and Scott-Knott test at 5% probability, analysing in a factorial scheme (5x3) with the concentrations and evaluation days.

RESULTS AND DISCUSSION

Glufosinate ammonium had greater inhibition on the mycelial growth of *S. sclerotiorum*, compared to the herbicides glyphosate and dicamba (Figure 1), except at the highest dose. The mycelial growth was completely inhibited with 10000 ppm glufosinate ammonium and reduced by 95% and 83% with glyphosate and dicamba. On average, *S. sclerotiorum* growth inhibition by glufosinate ammonium was 6 times greater than that by glyphosate and 4 times greater than that by dicamba at the three smallest doses. Inhibition by glufosinate reached 42% even at the lowest concentration, while for glyphosate and dicamba the inhibition was 5% and 13%, respectively.

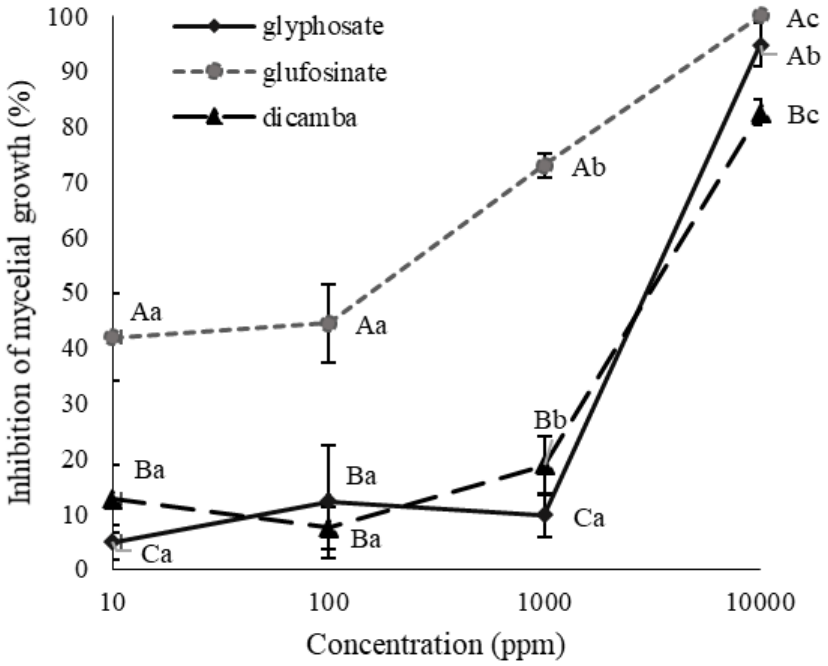

Figure 1. Inhibition of mycelial growth of *Sclerotinia sclerotiorum* at different concentrations of glyphosate, glufosinate ammonium and dicamba. Upper case letters compare herbicides within the dose factor, while lowercase letters compare doses within the herbicide actor. Averages followed by the same letter do not differ according to Scott-Knott test at 5% probability.

Not only was mycelial growth inhibited, but there was also a reduction in growth speed (Figure 2). The mycelial growth speed index with the addition of 10 ppm glufosinate was lower than that of the control, indicating the action of this herbicide even at lower doses. Compared to the control, the growth rate with 10 ppm glyphosate was 1 time lower, while the subsequent doses led to reductions by 1.1, 0.9 and 17.8 times. Similarly, the maximum reduction in growth speed caused by dicamba was 6.4 times that of the control.

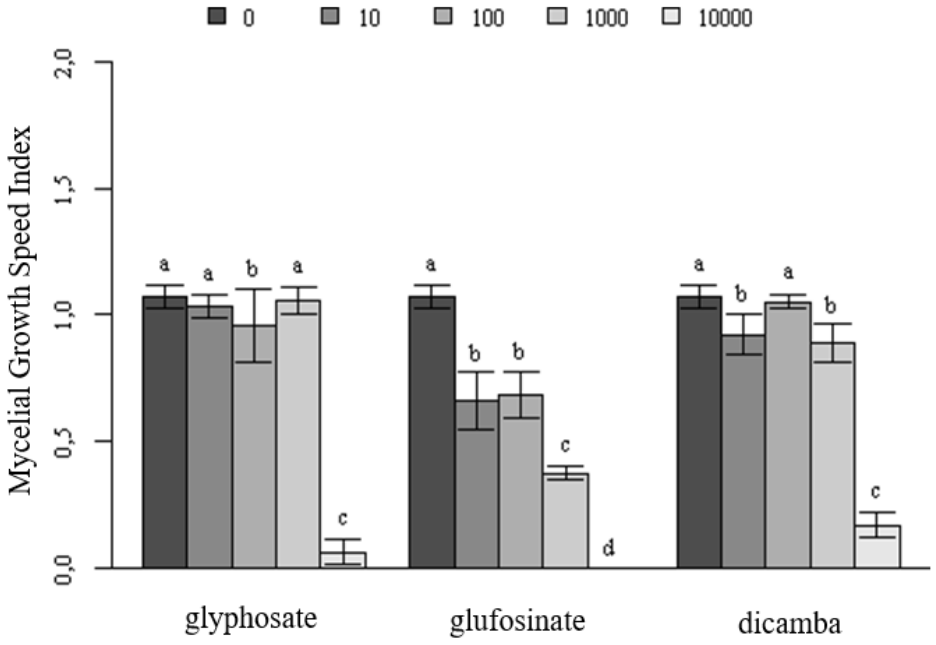

Figure 2. Mycelial growth speed index of *Sclerotinia sclerotiorum* at different concentrations (0.10, 100, 1000 and 10000 ppm) of glyphosate, glufosinate ammonium and dicamba. Averages followed by the same lowercase letter for each herbicide do not differ according to Scott-Knott test at 5% probability.

The results of the present study are in agreement with the mycelial inhibition of *S. sclerotiorum* reported by Lehner et al. (12), in which the herbicide glyphosate inhibited 84% at a concentration of 1000 mg. L^{-1}. Other herbicides have been described as inhibitors of this fungus, such as bentazon with 87% (12), chlorimuron-ethyl with 84% (9), diuron and paraquat (4). Lehner et al. (12) mentioned that glyphosate application in places with direct, no-till farming can have a negative influence on fungi that survive on crop remains or are present in the soil. This raises questions on the action of glufosinate under certain field conditions, given the high inhibition rate reported in this study.

Considering the control sample, the number of stipes decreases with time (Table 1), while the number of apothecia increases (Table 2), precisely due to the differentiation that occured. However, as the concentration of glyphosate or glufosinate ammonium increased, the number of stipes did not decrease but increased with time. The concentration of 10000 ppm glufosinate ammonium exemplifies this fact. Regardless of the concentration, all glyphosate treatments led to a significant increase in the number of stipes at 40 and 50 days.

Even though the interaction between concentration and days was not significant, the factors could be analyzed separately. Addition of glyphosate, regardless of the concentration, increased the number of stipes, as mentioned above, but reduced the number of apothecia (Table 2). Dicamba also reduced the formation of apothecia as the concentration increased.

Changes in the morphology of stipes and apothecia occurred since the lowest concentrations of the herbicides glyphosate, glufosinate ammonium and dicamba (Figure 3). Glufosinate ammonium stimulated the emission of stipes, as illustrated in Figure 3-A and Table 1; however, they did not differentiate into apothecia, with flattened and discoid ends, and resulted in sclerotia with numerous elongated stipes. Glyphosate led to drastic deformations at the highest concentrations resulting in abnormal apothecia with fused disk edges (Fig 3-G), without the characteristic presence from the apical orifice of the disk through which the ascospores are released. Absence of the cream color was also observed in abnormal apothecia by the application of dicamba, acquiring a dark beige color (Figure 3-D).

Considering the survival structures of *S. sclerotiorum*, the herbicides analyzed here affected the number of stipes, apothecia, or both. Although the action of trifluralin, lactofen, setoxydim, metribuzim and imazethapyr had not altered the number of stipes or apothecia, Vrisman et al. (16) found a reduction in the germination of sclerotia treated with these herbicides. In addition, the present study reports the formation of abnormal apothecia, showing drastic deformations in their morphology due to glyphosate, glufosinate and dicamba. Casale and Hart (3) had already reported similar deformations in apothecia treated with atrazine, including the absence of ascospores from these structures, which characterizes sterility. The herbicide simazine has also been described as an agent of deformations in the apothecia of this fungus (11).

Glyphosate acts on the EPSPs enzyme in plants and its action on microorganisms is attributed to the same enzyme present in oomycete, ascomycete and basidiomycete fungi (NANDULA, 2010). Although the mechanisms of action of glufosinate ammonium and dicamba are relatively well known, such action on fungi is not completely elucidated.

Table 1. Number of stipes on sclerotia of *Sclerotinia sclerotiorum* at 30, 40 and 50 days under different doses of the herbicides glyphosate, glufosinate ammonium and dicamba.

	Stipes								
	Glyphosate			Ammonium glufosinate			Dicamba		
ppm	30d	40d	50d	30d	40d	50d	30d	40d	50d
0	12.0 Aa	4.2 Ba	2.7 Ba	12.0 Aa	4.2 Ba	2.7 Ba	12.0 Aa	4.2 Ba	2.7 Ba
1	11.2 Aa	8.5 Ab	7.5 Ab	16.7 Aa	11.5 Aa	8.2 Aa	9.5 Aa	5.0 Aa	4.0 Aa
10	18.2 Aa	14.0 Ac	9.7 Ab	14.2 Aa	7.0 Aa	8.5 Aa	8.2 Aa	8.2 Aa	11.2 Ab
100	14.7 Aa	15.0 Ac	11.5 Ab	12.2 Aa	9.7 Aa	10.2 Aa	7.2 Aa	6.5 Aa	7.7 Ab
1000	15.2 Aa	19.5 Ac	12.2 Ab	17.0 Aa	23.7 Ab	23.5 Ab	7.7 Aa	9.2 Aa	9.2 Ab
10000	13.0 Aa	16.5 Ac	17.2 Ab	0.0 Aa	9.5 Ba	16.0 Ba	5.5 Aa	7.0 Aa	2.2 Ba
CV%	9.08			39.65			26.71		

Averages followed by the same uppercase letters in the row and lowercase letters in the column do not differ according to Scott-Knott test at 5% probability.

Table 2. Number of apothecia in sclerotia of *Sclerotinia sclerotiorum* under different doses of the herbicides glyphosate, glufosinate ammonium and dicamba.

	Apothecia											
	Glyphosate				Ammonium glufosinate				Dicamba			
ppm	30d	40d	50d	média	30d	40d	50d	média	30d	40d	50d	média
0	8.2	17.5	16.7	14.2a	8.2	17.5	16.7	14.2 a	8.2	17.5	16.7	14.2 a
1	3.0	6.7	5.0	4.9 b	7.2	13.0	11.0	10.4 b	10.2	12.0	8.2	10.1 b
10	3.5	7.2	5.7	5.5 b	5.0	11.7	9.2	8.6 b	5.0	7.2	3.7	6.5 c
100	3.2	5.7	7.0	5.3 b	5.0	10.0	9.5	8.3 b	4.7	7.0	6.2	6.0 c
1000	1.5	3.7	4.5	3.2 c	2.7	10.5	11.7	8.1 b	7.0	6.7	6.0	5.3 c
10000	0.5	2.0	3.2	1.9 d	0.0	0.0	2.5	0.8 c	0.7	1.7	3.2	1.9 d
CV%	40.18				38.77				48.61			

No significant interaction. Averages followed by the same lowercase letters in the column do not differ according to Scott-Knott test at 5% probability.

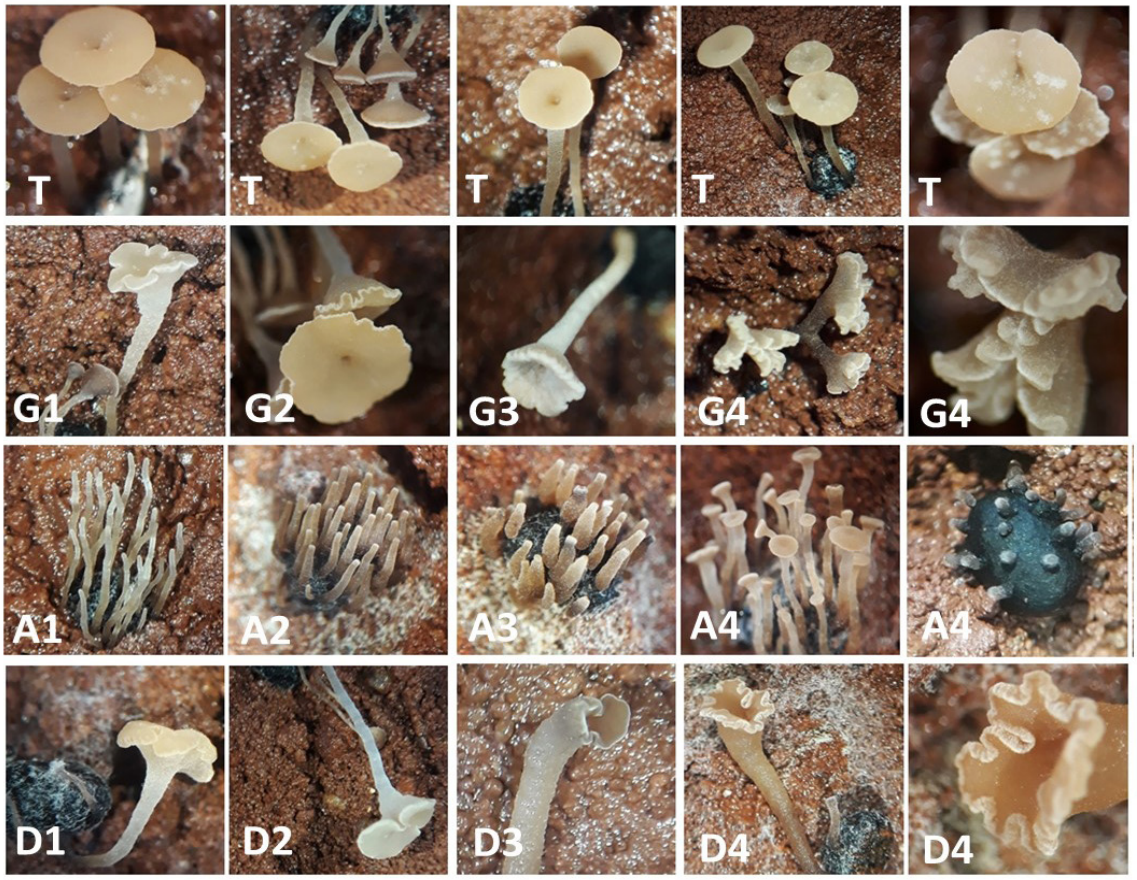

Figure 3. *Sclerotinia sclerotiorum* apothecia from the control treatment (T); deformations in apothecia subjected to glyphosate (G), glufosinate ammonium (A) and dicamba (D) at the concentrations of 10, 100, 1000 and 10000 ppm (1, 2, 3 and 4, respectively).

This is the first report on the inhibitory action of glufosinate ammonium and dicamba on *Sclerotinia sclerotiorum*. The relevance of these results is based on the wide range of hosts of this fungus and the rise in the use of pre-planting and post-emergence herbicides in genetically modified soybean cultivars (7,8).

Herbicides inhibited mycelial development, as well as the speed of mycelial growth and carpogenic development of *Sclerotinia sclerotiorum*. Even at low concentrations, glufosinate ammonium reduced the speed and inhibited the mycelial growth at higher levels, compared to glyphosate and dicamba. Herbicides stimulated the emission of sclerotia stipes, but reduced the number of sclerotia apothecia.

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