



# Influence of herbicides on the carpogenic germination of *Sclerotinia sclerotiorum* sclerotia

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**Abstract.** We investigated the influence on carpogenic germination of *Sclerotinia sclerotiorum* sclerotia of thirty-one registered herbicides commonly used in production of cereals, oilseeds, pulse, potato, and sugarbeets in southern Alberta, and of two unregistered herbicides. Compared to the untreated controls, none of the herbicides showed any stimulatory or inhibitory influence on the carpogenic germination of sclerotia, when incorporated in soil at the recommended field rates. These herbicides had little influence on the differentiation and development of apothecia—except for the triazine herbicides simazine and atrazine, which induced formation of secondary stipes on the primary stipes. Simazine caused the formation of cockscomb-like apothecia containing very few asci and ascospores, and atrazine caused balls of mycelia without asci to form on the tips of stipes. Abnormal apothecial morphogenesis was induced by simazine and atrazine at 25% to 50% of the recommended field rate (1500 g/ha).

**Keywords:** Apothecia; Carpogenic germination; Herbicides; *Sclerotinia sclerotiorum*.

## Introduction

*Sclerotinia* diseases caused by *Sclerotinia sclerotiorum* (Lib.) de Bary are a common problem of pulse and oilseed crops in western Canada (Martens et al., 1984); especially in crops grown under irrigation (Huang et al., 1988a, 1988b). In this region, pulses and oilseeds are grown in rotation with a variety of crops, including cereals, corn, potato, alfalfa, and sugarbeet. *Sclerotinia sclerotiorum* is thus exposed directly, and indirectly through residues from previous crops, to a variety of agrichemicals which are used in crop production on the prairies.

Ascospores produced from carpogenic germination of sclerotia are the primary inoculum which infects oilseed and pulse crops on the prairies—with the exception of sclerotinia wilt of sunflower, which is caused by mycelia from mycelial germination of sclerotia of *S. sclerotiorum* (Huang and Dueck, 1980). Carpogenic germination of sclerotia of *S. sclerotiorum* is affected by fungicides (Hawthorne and Jarvis, 1973; Jones, 1974; Dueck et al., 1983), herbicides (Liu, 1977; Cerkauskas et al., 1986; Radke and Grau, 1986; Teo et al., 1992), nematocides (Partyka and Mai, 1958), and fertilizers (Mitchell and Wheeler, 1990; Huang and Janzen, 1991; Huang and Sun, 1991). Previous studies have demonstrated that some herbicides, such as calcium cyanamide (Jones and Gray, 1973; Huang and Sun, 1991) and linuron (Radke and Grau, 1986), inhibited carpogenic germination of *S. sclerotiorum* sclerotia, but that others, such as trifluralin, pendimethalin,

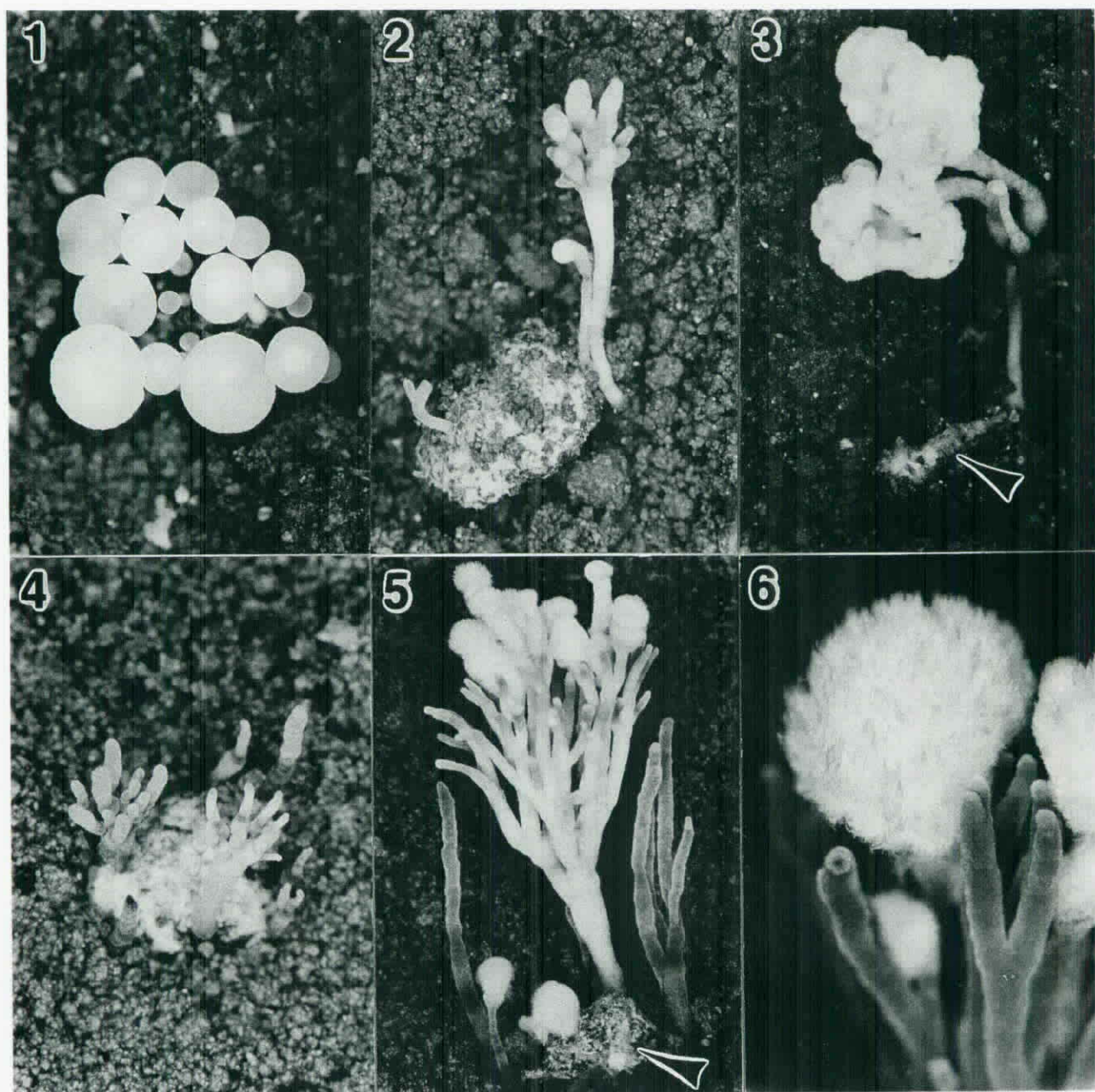
metribuzin, simazine, and atrazine, stimulated germination (Radke and Grau, 1986). Atrazine and simazine also caused formation of abnormal apothecia of *S. sclerotiorum* (Liu, 1977; Radke and Grau, 1986).

Several triazine herbicides, such as atrazine and simazine, are persistent in soils in Saskatchewan (Smith and Walker, 1989) and Alberta (Moyer and Blackshaw, 1993; Walker et al., 1983). The residues of several herbicides, including triazines, are capable of reducing subsequent crop yields (Moyer, 1992; Moyer and Blackshaw, 1993). Herbicides are widely used in the production of field crops in southern Alberta (Anonymous, 1993), and the influence of many of these herbicides on *S. sclerotiorum* was unknown. This study was initiated to determine whether herbicides commonly used on the prairies affect the carpogenic germination of *S. sclerotiorum* sclerotia.

## Materials and Methods

Thirty-three herbicides, including 31 registered for use on Canadian crops (e.g. cereals, pulses, oilseeds, corn, and potatoes) and two unregistered, experimental herbicides (Table 1) were assessed for their influence on carpogenic germination of *S. sclerotiorum* sclerotia. All herbicides were dissolved or suspended in 100 ml of distilled water and mixed with sandy clay loam soil (57% sand, 17% silt, 26% clay, 2% organic matter, pH 8.0) at the equivalent of the recommended field rate (assuming 2-cm penetration of the herbicide into soil). The herbicide-treated soil was shaken in a plastic bag for 20 min, equilibrated for 24 h,

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**Figures 1–6.** Influence of simazine (Figures 2, 3) and atrazine (Figures 4–6) on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*. The sclerotia were incubated on soil under fluorescent light for 3 (Figure 1), 5 (Figures 2, 3), 6 (Figure 4) and 9 (Figures 5, 6) weeks. Normal apothecia were formed on the sclerotium on untreated soil (Figure 3). Note multiple secondary stipes (Figures 2, 4, 5) and tips differentiated into cockscomb-like apothecia (Figure 3) or fuzzy balls of mycelia (Figure 6). Approximate magnifications: Figure 1, 5.4 $\times$ ; Figure 2, 5.6 $\times$ ; Figure 3, 5.9 $\times$ ; Figure 4, 4.9 $\times$ ; Figure 5, 4.9 $\times$ ; Figure 6, 13.2 $\times$ .

and then 50 g of the soil was put into each of three petri dishes. Atrazine, simazine, bromoxynil, and triallate were also tested at rates higher and lower than the recommended rates. Distilled water was used to adjust soil moisture to 16–17% by weight. The same soil without herbicide was used as the control.

Sclerotia of *S. sclerotiorum*, isolate sun-87, were harvested from cultures grown on potato dextrose agar (PDA) at 10°C for 8 weeks (Huang and Kozub, 1991), and placed on the soil (10 sclerotia per petri dish). The dishes were individually sealed with parafilm, incubated at 20°C un-

der fluorescent light ( $15.2 \mu E m^{-2} s^{-1}$ ) for 3 weeks, and examined for carpogenic germination of sclerotia and production of apothecia. Sclerotia that produced stipes without disc differentiation were kept under the same conditions for up to 9 weeks to observe the development of normal or abnormal apothecia. Free-hand sections of normal and abnormal apothecia were stained with cotton blue in lactophenol and examined for asci and ascospores in the hymenium layer under a compound microscope. Sclerotia that failed to germinate and produce stipes in 3 weeks were surface-sterilized in 70% ethanol for 90 s,

**Table 1.** Influence of herbicides on carpogenic germination of *Sclerotinia sclerotiorum* sclerotia.

Herbicide	Rate		Carpogenic germination	
	g/ha	(ppm)	(%)	Apothecia/sclerotium
<b>Test I</b>				
2-4,D	560	(2.80)	100	5.1a <sup>b</sup>
Imazethapyr (Pursuit)	100	(0.50)	100	5.0a
Thifensulfuron (Refine)	30	(0.15)	100	4.9a
Trifluralin (Treflan)	1100	(5.50)	100	4.9a
Eptam (EPTC)	3500	(17.50)	100	4.2a
Atrazine	1500	(7.50)	100	0.0b
Control	0	(0.00)	100	5.0a
<b>Test II</b>				
Sethoxydim (Poast)	300	(1.5)	90a <sup>b</sup>	3.0a <sup>b</sup>
Bentazon (Basagran)	1100	(5.5)	87a	2.6a
Flurtamone (Benchmark) <sup>a</sup>	1000	(5.0)	80a	3.0a
Bromoxynil (Torch)	400	(2.0)	70ab	2.2a
Glyphosate (Roundup)	1000	(5.0)	67ab	2.2a
Diclofop methyl (Hoegrass)	800	(4.0)	40b	2.7a
Control	0	(0.0)	97a	3.1a
<b>Test III</b>				
Linuron (Lorox)	2000	(10.0)	87a <sup>b</sup>	2.9a <sup>b</sup>
Cyanazine (Bladex)	2500	(12.5)	70ab	2.9a
Clopyralid (Lontrel)	200	(1.0)	67ab	2.0ab
Dicamba (Banvel)	140	(0.7)	57bc	1.9ab
Simazine	2500	(12.5)	50bc	1.6b
Metribuzin (Sencor)	560	(2.8)	40c	1.3b
Control	0	(0.0)	73ab	2.0ab
<b>Test IV</b>				
Linuron (Lorox)	2000	(10.00)	100a <sup>b</sup>	8.9a <sup>b</sup>
Clopyralid (Lontrel)	200	(1.00)	100a	7.4ab
Imazamethabenz (Assert)	500	(2.50)	100a	6.5bc
Dicamba (Banvel)	140	(0.70)	100a	5.8bcd
Ethylmetribuzin (Tycor) <sup>a</sup>	2000	(10.00)	100a	5.8bcd
Ethalfuralin (Edge)	1100	(5.50)	100a	5.3cde
Ethametsulfuron (Muster)	25	(0.13)	100a	5.1cdef
Triallate (Avadex)	2000	(10.00)	100a	4.7defg
Metolachlor (Dual)	2000	(10.00)	100a	4.5defg
Fluazifop-P (Fusilade)	125	(0.63)	100a	4.4defg
Difenzoquat (Avenge)	850	(4.25)	100a	3.7efg
MCPA Ester	560	(2.80)	100a	3.5fg
Tralkoxydim (Achieve)	250	(1.25)	100a	3.0g
Phenmedipham (Betanal)	1000	(5.00)	93ab	4.7defg
Paraquat (Sweep)	500	(2.50)	90b	3.1g
Control	0	(0.00)	87b	3.0g
<b>Test V</b>				
Metribuzin (Sencor)	560	(2.8)	100a <sup>b</sup>	6.0a <sup>b</sup>
Diclofop methyl (Hoegrass)	800	(4.0)	97a	6.5a
Glyphosate (Roundup)	1000	(5.0)	93a	5.8a
Bromoxynil (Torch)	400	(2.0)	57b	3.5b
Triallate (Avadex)	2000	(10.0)	47b	2.0c
Control	0	(0.0)	97a	5.1a

<sup>a</sup> Herbicides that are not registered in Canada.

<sup>b</sup> Means within a column and a test followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% level.

**Table 2.** Influence of various rates of atrazine, simazine, bromoxynil, and triallate on carpogenic germination of *Sclerotinia sclerotiorum* sclerotia.

Herbicide	Rate		Carpogenic germination <sup>a</sup>	
	g/ha	(ppm)	(%)	Apothecia/sclerotium
<b>Test I</b>				
Atrazine	75	(0.38)	98ab <sup>b</sup>	4.2a <sup>b</sup>
Atrazine	150	(0.75)	98ab	4.7a
Atrazine	375	(1.88)	100a	5.4a <sup>c</sup>
Atrazine	750	(3.75)	98ab	3.7a <sup>c</sup>
Atrazine	1125	(5.63)	95ab	4.7a <sup>c</sup>
Atrazine	1500	(7.50)	100a	5.2a <sup>c</sup>
Atrazine	3000	(15.00)	90abc	3.5a <sup>c</sup>
Atrazine	15000	(75.00)	85c	3.6a <sup>c</sup>
Simazine	75	(0.38)	95ab	3.8a
Simazine	150	(0.75)	98ab	3.6a
Simazine	375	(1.88)	98ab	4.3a <sup>c</sup>
Simazine	750	(3.75)	100a	3.3a <sup>c</sup>
Simazine	1125	(5.63)	98ab	3.3a <sup>c</sup>
Simazine	1500	(7.50)	88bc	3.3a <sup>c</sup>
Simazine	3000	(15.00)	85c	3.1a <sup>c</sup>
Simazine	15000	(75.00)	95ab	3.4a <sup>c</sup>
Control	0	(0.00)	100a	4.0a
<b>Test II</b>				
Bromoxynil	20	(0.1)	98a <sup>b</sup>	3.1abcd <sup>b</sup>
Bromoxynil	40	(0.2)	92a	2.5cdef
Bromoxynil	100	(0.5)	92a	3.7a
Bromoxynil	200	(1.0)	98a	2.7abcde
Bromoxynil	300	(1.5)	97a	3.7a
Bromoxynil	400	(2.0)	98a	2.7abcde
Bromoxynil	800	(4.0)	93a	2.5cdef
Bromoxynil	4000	(20.0)	32c	2.7bcde
Triallate	100	(0.5)	98a	2.6cdef
Triallate	200	(1.0)	95a	2.8abcde
Triallate	500	(2.5)	95a	2.8abcde
Triallate	1000	(5.0)	95a	2.3def
Triallate	1500	(7.5)	90a	2.3def
Triallate	2000	(10.0)	95a	2.0ef
Triallate	4000	(20.0)	68b	1.7f
Triallate	20000	(100.0)	0d	0.0g
Control	0	(0.0)	98a	3.4abc

<sup>a</sup> Data on carpogenic germination are based on average of two experiments in each test.

<sup>b</sup> Means within a column and a test followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% level.

<sup>c</sup> Sclerotia produced malformed stipes and apothecia.

placed on PDA at room temperature for 1 week, and examined for viability.

Analyses of variance (ANOVAs) for a complete randomization design were conducted on the data from each test to examine the influence of herbicide treatments on the proportion of sclerotia which germinated carpogenically and the number of apothecia that developed. All statistical analyses were conducted with SAS software (SAS Institute Inc., 1989).

## Results

In herbicide-free soil, sclerotia of *S. sclerotiorum* germinated carpogenically to produce stipes and form mature apothecia (Figure 1) after a 3-week incubation under light. When sclerotia were placed on herbicide-treated soil, 28 of the 33 herbicides showed no significant ( $P>0.05$ ) stimulatory or inhibitory influence on carpogenic germination of sclerotia—compared with the untreated control (Table 1). Diclofop methyl, dicamba, simazine, metribuzin, and triallate reduced carpogenic germination of sclerotia, but the results were inconsistent between experiments.

At less than the recommended rates, neither atrazine nor simazine affected germination of sclerotia or production of apothecia of *S. sclerotiorum* (Table 2). Atrazine and simazine caused only a slight reduction in carpogenic germination of sclerotia even when the rate was increased to two times (3000 g/ha) or 10 times (15000 g/ha) higher than the recommended rate (1500 g/ha) (Table 2). Bromoxynil significantly reduced carpogenic germination of sclerotia when applied at 10 times (4000 g/ha) higher than the recommended rate (400 g/ha). Triallate at double the recommended rate slightly reduced carpogenic germination of sclerotia, and at 10 times the recommended rate, all sclerotia were killed (Table 2).

Apothecia produced on sclerotia placed on herbicide-amended soil were normal for all the herbicides tested except atrazine and simazine which produced malformed apothecia (Figures 2–6). The minimum rate which induced formation of abnormal apothecia was 50% (750 g/ha) of the recommended rate of atrazine (1500 g/ha), and at 25% (375 g/ha) of the recommended rate of simazine (1500 g/ha) (Table 2). Sclerotia in untreated soils germinated carpogenically to produce primary stipes. The tip of each stipe differentiated and developed into a disc-shaped head or apothecium (Figure 1) bearing a hymenium with asci and ascospores. For sclerotia on simazine- or atrazine-treated soil, the tip of the primary stipes continued to elongate and branch without disc differentiation, resulting in the formation of multiple secondary stipes (Figures 2,4,5). Prolonged incubation of these sclerotia on simazine-treated soil often resulted in the formation of abnormal apothecia with convoluted or cockscomb-like discs on the secondary stipes (Figure 3). On atrazine-treated soil, fuzzy, ball-shaped structures developed on the secondary stipes (Figures 5–6). Microscopic examination of the cockscomb-like apothecia (Figure 3) showed very few asci with ascospores in the hymenium layer. The fuzzy, ball-shaped structures (Figure 6) consisted of mycelia and were completely sterile.

## Discussion

Although inhibition of carpogenic germination and development of the apothecium of *S. sclerotiorum* by linuron (Radke and Grau, 1986) and calcium cyanamide (Huang and Sun, 1991) has been reported, the 33 herbicides tested

in this study had little detrimental influence on carpogenic germination of this pathogen. Also, herbicides, except atrazine and simazine, did not affect the development of apothecia. Formation of malformed apothecia was not observed with other triazine herbicides, cyanazine, and metribuzin.

Our finding that trifluralin had no influence on apothecium production by *S. sclerotiorum* is in agreement with Cerkauskas et al. (1986), but in contrast with the report of Radke and Grau (1986) that described stimulation of carpogenesis of the pathogen by this herbicide. Radke and Grau (1986) also reported stimulation of carpogenesis of *S. sclerotiorum* by simazine and metribuzin, which we did not observe. Trifluralin, a dinitroaniline herbicide widely used for weed control in oilseed and pulse crops in southern Alberta, has been reported to make beans, cotton, and sugarbeets more vulnerable to attack by the pathogens *Rhizoctonia* and *Fusarium* (Altman and Rovira, 1989), and solanaceous vegetables more susceptible to verticillium wilt caused by *Verticillium dahliae* (Altman, 1985). Altman and Rovira (1989) found that trifluralin at 0.5 to 2.0 ppm—equivalent to 9 to 36% of the recommended rate we used—had no influence on mycelial growth of *Fusarium solani* f. sp. *phaseoli*, but stimulated the production of macroconidia and enhanced the germination of chlamydospores. Thus, sensitivity of microorganisms to a herbicide may vary with species.

Herbicides have been shown to induce formation of abnormal apothecia in discomycetes. Moore-Landecker (1972) reported that 2,4-D at 500 ppm induced formation of abnormal, sterile apothecia in *Pyronema domesticum* (Sow. ex Gray) Saccardo. Our study showed that treatment of sclerotia of *S. sclerotiorum* at 560 g/ha (2.8 ppm) produced normal apothecia (Table 1). Whether 2,4-D can induce formation of abnormal apothecia of *S. sclerotiorum* at high rates remains unknown.

Our study confirms previous reports (Liu, 1977; Radke and Grau, 1986) that the triazine herbicides—atrazine and simazine—enhanced branching of stipes and induced malformation of the apothecia of *S. sclerotiorum*. The triazine herbicides inhibit the growth of higher plants by blocking photosynthesis (Ashton and Crafts, 1981). The formation of stipe initials is not light-dependent (Purdy, 1956), but the tips of stipes are photosensitive and phototropic (Le Tourneau, 1979). Since light is essential to the differentiation and complete expansion of the apothecial disc of *S. sclerotiorum* (Henson and Valteau, 1940; Purdy, 1956; Phillips, 1987), it is possible that the triazine herbicides may interfere with photochemical interactions required during disc differentiation.

This study reveals that, when applied at recommended rates, most of the herbicides commonly used for crop production in southern Alberta are not toxic to sclerotia of *S. sclerotiorum*. The study also suggests that residues of atrazine and simazine from previous crops, such as corn, may reduce the ascospore inoculum in the field by causing malformed or sterile apothecia—even at 25% to 50% of the normal rates.

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## 殺草劑對菌核病菌菌核子囊盤發芽之影響

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本研究旨在探討 31 種在加拿大 Alberta 省常用殺草劑及兩種尚未註冊的殺草劑對菌核病菌 (*Sclerotinia sclerotiorum*) 菌核子囊盤發芽 (carpogenic germination) 之影響。試驗結果顯示這 33 種殺草劑於土中含量與田間使用量相同的情況下，均無明顯地促進或抑制菌核發芽現象，而且在這 33 種殺草劑中，只有 atrazine 和 simazine 會影響子囊盤分化，Simazine 能夠促進核盤柄 (stipes) 的再分枝，並於有些分枝頂端形成雞冠狀的畸型子囊盤，內含少量子囊及子囊孢子，Atrazine 也會促進核盤柄的再分枝，而於有些分枝頂端產生棉鈴狀的菌絲束，不含任何子囊及子囊孢子，將這兩種殺草劑使用量減為正常用量的 25 至 50%，仍可導致畸型子囊盤形成的現象，Triallate 殺草劑如超出正常使用量的十倍 (即 20000 g/ha) 可使菌核致死而喪失發芽能力。

**關鍵詞：**子囊盤；子囊盤萌發；殺草劑；*Sclerotinia sclerotiorum*。