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SURVIVAL OF BURIED *Sclerotinia sclerotiorum* SCLEROTIA IN UNDISTURBED SOIL

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SUMMARY

Sclerotia of *S. sclerotiorum* are surviving structures and the most important source of inoculum in sunflower fields. There are few studies about sclerotia survival in uncultivated soil and in accordance to that our objective was to establish the effect of burial depth on sclerotia longevity and the ability of carpogenic germination. In the trial (2009-2011) sclerotia were buried at the depth of 5, 10 and 30 cm and recovered every year in June. In laboratory sclerotia survival and carpogenic germination were examined. Our results showed that a large percentage of sclerotia survive at least three years under suitable conditions of temperature and moisture. In the case of continuous flooding (2011) sclerotia placed shallow in the soil (5 cm) were completely destroyed. Achieved results suggest that in undesturbed soil sclerotia placed deeper in the soil (10 and 30 cm) stay alive longer than those in upper soil (5 cm). Regardless of the burial depth sclerotia were able to produce apothecia under laboratory conditions. Resident saprofitic soil fungi (*Aspergillus* sp., *Fusarium* sp., *Mucor* sp. and mycoparasitic *Conyothirium* sp.) have been isolated equally from alive and deceyed sclerotia, but still less from the viable one.

Key words: ***S. sclerotiorum* sclerotia, longevity of sclerotia, carpogenic germination, undisturbing soil**

INTRODUCTION

Sclerotinia sclerotiorum is a cosmopolitan non-specific pathogen on more than 400 plant species, both cultivated and wild (Boland and Hall, 1994; Jurković and Culak, 1997; Vrandečić *et al.,* 2003). In Croatia *S. sclerotiorum* is one of the main destructive pathogens on sunflower and causes at least three disease types: basal stem rot, rot of upper stem part and head rot. The first two diseases are usually known as white stem rot. All types of diseases have strong impact on yield quantity and quality during cool and wet seasons.

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The major source of inoculum are sclerotia, structures which can remain viable about 4-5 years in soil (Adams and Ayers, 1979) or even longer (Harvey *et al.*, 1995). Sclerotia are deposited on soil surface, below soil level or deeper in soil. But little is known about vertical distribution of sclerotia in soil.

Depending on sclerotia depth, cultural practices, environmental conditions and soil micopopulation sclerotia longevity is different (Adams, 1975; Kurle *et al.*, 2001; Mueller *et al.*, 2002; Duncan *et al.*, 2006). However, results are often very confusing. Merriman *et al.* (1979) found that percentage of survived sclerotia and apothecia production from bean and lettuce depend on the location and depth of sclerotia burial. Deep plowing decreased density of sclerotia in soil, apothecial formation and sclerotia infection (Mueller *et al.*, 2002). Contrary, Kurle *et al.* (2001) reported that plowing is not effective in soybean stem rot caused by *S. sclerotiorum*. Uncultivated soils have higher microbial activity therefore sclerotial degradation should be higher (Workneh and Young, 2000). In unadequate environmental conditions sclerotial survival and germination are diminished. Moore (1949) investigated three flooded soil types and determined that sclerotia were completely destroyed within 24-45 days.

In extensive literature there are few studies about sclerotia survival in undisturbed soil. Since weed and volunteer plants are the hosts for *S. sclerotiorum* (Jurković and Culek, 1977; Vrandečić *et al.*, 2003) and grow continuously on uncultivated areas this research can be helpful in better understanding of the necessity of their control.

In accordance to that our objective was to study effect of burial depth in undisturbed soil on sclerotia longevity and ability of carpogenic germination.

MATERIAL AND METHODS

Sclerotia of *S. sclerotiorum* originating from sunflower plants were collected in summer 2008 (August) and devided into 12 samples, each containing 30 g of sclerotia of different size as it was found in the field. All samples were place in plastic net-bags, mixed with choped soil and buried for a longer period of time in uncultivated soil at the depth of 5, 10 and 30 cm. The trial started in 2008 and was perfomed during three years.

The first group of samples were dug up after 10 months (June 2009), the second group after 22 months (June 2010) and the third group of samples after 34 months (June 2011). Recovering of sclerotia samples was in June since weather conditions (temperature and precipitation) in June and at the begining of July support sunflower infection.

In the laboratory sclerotia were washed under tap-water (2 h), surface sterilised (97% ethil alcohol for 2 min.), rinsed twice in distilled water and air-dried. In Petri dishes (Ø 90 mm) 4 × 10 sclerotia from each depth were set up on threefold filter paper saturated with distilled water and kept on laboratory desk at 22±1°C upon

naturally light/dark regime. In every Petri dish there were 5 small (3-4 mm) and 5 larger (≥4 mm) sclerotia. Filter paper moisture was maintained daily with distilled water.

Inspections under stereo microscope (Olimpus SZX9) were done every three days during 27 days.

RESULTS AND DISCUSSION

Summarized results are presented in Table 1.

Regardless of their size and burial depth the highest percentage (99.2) of all sclerotia survived 10 month burial. Only one larger sclerotia (≥4 mm) buried at the depth of 5 cm was missing.

Table 1: Effect of burial depth on longevity and carpogenic germination of *S. sclerotiorum* sclerotia

Year	Depth (cm)	Recovered sclerotia*	Missing sclerotia	% sclerotia with stipes**		% sclerotia with apothecia**
				3rd day	27th day	
2009	5	39	1	25.6	51.2	2.6
	10	40	0	25.0	52.5	12.5
	30	40	0	62.5	97.5	10.0
2010	5	36	4	2.8	50.0	47.2
	10	36	4	8.3	58.3	55.5
	30	38	2	10.5	92.1	76.3
2011	5	0	0	0	0	0.
	10	6	36	83.3	100.0	16.7
	30	14	26	42.8	71.4	21.4

* recovered sclerotia is the number of total (40) sclerotia per depth taken into lab examination
** sclerotia with stipes and sclerotia with apothecia are given as the percentage of number of recovery sclerotia

First stripes, as a wart or very short thread about 1-2 mm long, could be seen under stereo microscope on the 3rd day. This was accepted as a sign of sclerotia survival. Percentage of alive sclerotia continued to increase over the time and on the 27th day ranged from 51.2 (5 cm) to 97.5 (30 cm). Stripes became longer and reached up to 10 mm. Larger sclerotia produced many stripes, on average 15 per sclerotia, small ones produced fewer stripes (1-3 on average). Despite numerous stripes apothecia with asci and ascospores were infrequent.

Results achieved in 2010 (22 months burial) showed little difference in total percentage of alive sclerotia. It varied from 50.0%, 58.3% to 90.0% for burial depth of 5, 10 and 30 cm, respectively. Appearance of stripes establishesd on the 3rd day was a bit lower in comparison to the previous year. However, till the end of trial the average number of developed stripes was not significantly decreased. Large number of apothecia with developed asci arised, contrary to 2009 results.

During the next year (June 2010-June 2011) without exception sclerotia buried at 5 cm were missing and the percentage of sclerotia buried at 10 and 30 cm significantly decreased. Percentage of recovered sclerotia was only 6 (10 cm) and 14 (30 cm) and most of them were parasites. According to the data obtained by Moore (1949) sclerotia will be completely decayed after 24-45 days in flooded soil at temperature 22-24°C. In our case it occured that soil was flooded continually for one month as a consequence of extreme precipitation in May (162 mm) and June (273 mm) 2010 and average soil temperature at 5 cm was 19.2°C in May and 25.4°C in June.

Our study showed the development of stripes in a short time. The very first sign of stripe occurring was visible on the 3rd day and their growth was completed in 10 to12 days when they measured 10 mm.

We can not explain why apothecia were rare in 2009 and abundant in 2010. Low preconditioning temperature and continuously high moisture are crucial for carpogenic germination (Huang and Kozub, 1991; Dillard *et al.,* 1995; Harvey *et al.,* 2005; Wu and Subbarao, 2008; Mila and Yang, 2008, Venette, www.ndsu.nodak.edu/plantpath/sclero.) but since we did not have any preconditioning treatment this could be the reason. In addition, we suppose, burial duration, together with other factors, can effect carpogenic germination (Sun and Yang, 2000; Kurle *et al.,* 2001; Duncan *et al.,* 2005).

In our trial over 50% of sclerotia buried at 10 cm and over 90% buried at 30 cm remained alive during 22 months and can produce apothecia. On the other hand, Kurle *et al.* (2001) reported that sclerotia viability decreases in deeper soil under chisel plow and no-tillage cultivation system and sclerotia will carpogenically germinate within upper 5 cm of soil.

Saprofitic fungi, mostly from genera *Aspergillus*, *Fusarium* and *Mucor* have been found equally on the remaining and destroyed sclerotia. In 2011 *Coniothyrium* spp., an important mycoparasite of sclerotia of *S. sclerotiorum, S. minor, S. trifoliorum, Botrytis cinerea* and *B. fabae* (Cook and Baker, 1983) was isolated especially from destroyed sclerotia. However, sclerotia degradation in deeper soil (10 and 30 cm) due to resident soil microogranisms was lower in comparison to sclerotia at 5 cm. Our findings are in agreement with statement by Cook *et al.* (1975) but not with report by Workneh and Young (2000).

This study confirms the effect of temperature, moisture and also burial depth on longevity of *S. sclerotiorum* sclerotia. If the environmental conditions are favourable sclerotia will undoubtely survive during almost three years even they are deposited deep in the soil. At the same time achieved results indicate that sclerotia deeper in undisturbed soil (10 and 30 cm) stay alive for a longer period of time than those at lower depth (5 cm). It might be connected with weak activity of resident microorganisms in deeper layer of undisturbed soil. Sclerotia which survived under laboratory conditions are also convenient for carpogenic germination.

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