J. Mycopathol, Res, **49(1)** : 171—173 (2011)

A faster technique to produce ascospores of *Sclerotinia sclerotiorum* in laboratory

SUSANTA BANIK AND PRATIBHA SHARMA[1]
Department of plant pathology, SASRD, Nagaland University, Medziphema 797 106
[1]Division of Plant Pathology, IARI, Pusa Campus, New Delhi 110 012
E-mail-susant20@rediffmail.com

Received : 23.11.2009 Accepted : 21.12.2010 Published : 25.04.2011

Sclerotinia sclerotiorum (Lib.) de Bary, one of the most omnivorous and successful plant pathogenic fungi affects more than 400 plant species belonging to 75 families. It brings about enormous crop losses attacking several vegetables, oilseeds and other crops. The pathogen produces sclerotia as compact, hard mass of hypha resistant to adverse weather conditions and can survive in soil for long years making it difficult to control. In crop season these sclerotia can produce apothecia and ascospore which play important role in initiating infection as well as dispersal. It is imperative to produce these ascospores for further research in management aspect of the pathogen. Considering this, a study was undertaken to devise a technique for laboratory production of ascospores. Mature sclerotia were incubated for time durations ranging from 10 to 60 days in different quantities of sterile and distilled water (0, 5, 10, 15, 20 and 25 ml) contained in sterile conical flask at 15 °C. Carpogenic germination by apothecia formation was first seen 20 days after incubation (DAI) in all the treatments except in the one without water and the highest germination (40.3%) at 20 DAI was recorded with 10 ml water. However, maximum carpogenic germination (90.7%) was recorded 60 DAI with 10 ml water. Statistically, germination % of sclerotia of 50 DAI and 60 DAI were non-significant. Incubation of sclerotia with higher quantity of water was found inhibitory to apothecia formation. As compared to production of ascospores from sclerotia in other methods which required 90 days, the present method can save 40 days in the attempt to produce abundant ascospores for various research activities

Key words: *Sclerotinia sclerotiorum,* ascospore production, laboratory

INTRODUCTION

The pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary is a soil-inhabiting pathogen and considered one of the most omnivorous and successful plant pathogens. It affects more than 408 plant species in 75 families (Boland and Hall, 1994). It does the most damage to vegetables and oilseed species. Millions of dollars are lost each year due to reduced crop yields and quality of crops that are susceptible to this pathogen. Although the genus *Sclerotinia* is characterized by the production of apothecia and sclerotia, and the absence of conidia, the disease cycles associated with these fungi differ significantly. *Sclerotinia sclerotiorum* is considered an opportunistic invader of senescent or dead foliar and floral tissues (Andrews, 1992), and the epidemiology of disease is primarily associated with carpogenically-germinated sclerotia that produce apothecia, which release ascospores. This carpogenic germination is favored by optimum temperature conditions of 15°C (Hao *et al.*, 2003). Germinating ascospores colonize senescing or dead flower petals in the phyllosphere and after colonizing such nutrient sources; the pathogen invades adjoining living tissue and initiates disease (Abawi and Grogan, 1975; Abawi *et al*, 1975; McLean, 1958). In some crops, sclerotia of *S. sclerotiorum* in the soil can germinate myceliogenically and infect nearby plant tissue directly (Purdy, 1979; Tu, 1989). It is im-

perative to produce these ascospores aseptically in the laboratory for further research in various aspect of the pathogen. It was observed in our laboratory that these sclerotia germinated only three months after formation in the left-out-Petri plates where the fungus was allowed to grow. As the time required is quite long, a need has been felt to undertake some work to hasten the process. Considering this, a study has been undertaken to devise a faster technique for laboratory production of ascospores.

MATERIALS AND METHODS

Carpogenic Germination of sclerotia and ascospore production

Carpogenic germination of sclerotia of *S. sclerotiorum* might take place on left out PDA plates but it required about three months after sclerotia formation. A quick method of carpogenic germination was standardized by keeping surface sterilized sclerotia on different quantities of water contained in conical flask for different incubation periods.

Germination studies were carried out in water to find out optimum period and amount of water for maximum sclerotia germination. Mature sclerotia of 3-4 mm size were put in different quantities (0, 5, 10, 15, 20 and 25 ml) of distilled and sterilized water in 100 ml autoclaved conical flasks. They were plugged with non-absorbent sterilized cotton and incubated at 15°C under alternate light and dark conditions. Data for germination of sclerotia were recorded at 10 days interval up to 90 days. The maximum germination reached in 60 days with 10 ml of water and there was no increase after it. Apothecia produced as a result of carpogenic germination were the source of ascospores. Ascospores were harvested by crushing the apothecia in sterile distilled water. The data regarding role of water on carpogenic germination were noted.

RESULTS AND DISCUSSION

The data presented in Table 1 revealed that maximum carpogenic germination of 90.7% was observed in conical flasks with 10 ml of water after 60 days of incubation. However, 40.3% carpogenic germination was obtained 20 days after incubation at 10 ml of water. All the other quantities of water (lower and higher than 10 ml) showed significantly less carpogenic germination. Sclerotia also germinated in absence of water showing only 5% germination 60 days after incubation (DAI). The minimum time of incubation required for sclerotial germination through apothecia production was 20 days for all the treatments with water. Germination of 50% was achieved after 30 days in 10 ml of water. Though highest % of germination was achieved 60 days after incubation in all the treatments, it was

Table 1 : Carpogenic germination of sclerotia *S. sclerotiorum* under different quantities of water *in vitro*

Water (ml)	% Carpogenic germination at different days after incubation (DAI)*						Mean
	10	20	30	40	50	60	
0	0 (4.0)	0 (4.0)	0 (4.0)	0 (4.0)	4.7	5.0	6.9
				(12.4)	(12.7)		
5	0 (4.0)	14.7	24.7	40.0	48.7	50.0	30.8
		(22.5)	(29.8)	(39.2)	(44.0)	(45.0)	
10	0 (4.0)	40.3	50.0	75.0	90.0	90.7	48.7
		(39.4)	(45.0)	(60.0)	(71.6)	(72.3)	
15	0 (4.0)	25.3	30.3	45.0	54.0	54.7	35.2
		(30.2)	(39.2)	(42.1)	(47.9)	(47.7)	
20	0 (4.0)	15.0	19.7	30.0	39.7	40.3	27.5
		(22.7)	(26.3)	(33.1)	(39.0)	(39.4)	
25	0 (4.0)	6.3	10.3	15.3	15.0	15.0	17.1
		(14.4)	(18.7)	(23.0)	(22.8)	(22.8)	
Mean	4.0	22.2	27.2	33.6	39.6	39.9	

*Observation on 25 sclerotia in each of three replications Figures in parentheses are arc sin transformed values

	CD at 5%	SEm
Incubation period	: 1.28	0.45
Water	: 1.65	0.52
Incubation period × water	: 3.13	1.11

actually 50 days after incubation as the data under two DAI were statistically non-significant. Therefore, 50 DAI could be regarded to give highest % carpogenic germination. Thus, compared to other methods of carpogenic germination (keeping the sclerotia in Petriplate) which required three months (90 days), the present method was found to hasten the process of getting carpogenic germination by 40 days.

It is known from the literature that carpogenic germination of *S. sclerotiorum* sclerotia were affected significantly by soil moisture and temperature. The number of stipes produced by one sclerotium also varied significantly depending on the moisture (Hao *et al.*, 2003). With adequate soil moisture, apothecia were reported appearing in natural conditions in 10 days under average daily temperature of about 20°C (Sun and Yang, 2000). However, higher and lower soil moisture reduced carpogenic germination which corroborated with our finding. Germination rate of sclerotia of *S. sclerotiorum* after 2 to 8 weeks in irrigated soil on the surface or buried at a depth of 5 cm was significantly lower than that for sclerotia maintained in dry soil at the same depths. No sclerotia of *S. sclerotiorum* germinated after 3 weeks, after recovery from flooded soil with mean soil temperatures ranging from 30 to 33°C (Matheron and Porchas, 2005). In the present investigation optimum moisture level for carpogenic germination was not expressed in terms of metric potential, however, the optimum level was reported in the range of –0.03 or –0.07 MPa (Hao *et al.*, 2003).

REFERENCES

Abawi, G. S. and Grogan, R. G. 1975. Sources of primary inoculum and effects of temperature and moisture on infection of beans by *Whetzelinia sclerotiorum*. *Phytopathology*. **65:** 300-309.

Abawi, G. S.; Polach, F. J. and Molin, W. T. 1975. Infection of bean by ascospores of *Whetzelinia sclerotiorum*. *Phytopathology*. **65:** 673-678.

Andrews, J. H. 1992. Biological control in the phyllosphere. *Ann. Rev. Phytopathol.* **30:** 603-635.

Boland, G. J. and Hall, R. 1994. Index of plant hosts of *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology*. **16:** 93-108.

Hao, J,J.; Subbarao, K. V. and Duniway, J. M. 2003. Germination of *Sclerotinia minor* and *S. sclerotiorum* sclerotia under various soil moisture and temperature combinations. Phytopathology, **93**: 443-450.

Matheron, M. E. and Porchas, M. 2005. Influence of soil moisture and temperature on eruptive germination and viability of sclerotia of *Sclerotinia minor* and *S. sclerotiorum*. *Plant Disease*, **89:** 50-54.

McLean, D. M. 1958. Some experiments concerned with the formation and inhibition of apothecia of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Plant Dis. Reptr.* **42:** 409-412.

Purdy, L. H.1979. *Sclerotinia sclerotiorum*: history, diseases and symptomatology, host range, geographic distribution, and impact. *Phytopathology*. **69:** 875-880.

Sun, P. and Yang, X. B. 2000. Light, temperature and moisture effects on apothecium production of *Sclerotinia sclerotiorum*. *Plant Disease*, **84:** 1287-93.

Tu, J. C. 1989. Modes of primary infection caused by *Sclerotinia sclerotiorum* in navy bean. *Microbios*. **57**: 85-91.