PRODUCTION AND INTER-RELATIONSHIP OF TWO TYPES OF SECONDARY SPORIDIA OF *NEOVOSSIA INDICA*

H. S. DHALIWAL* and D. V. SINGH**†

International Wheat and Maize Improvement Centre (CIMMYT), Lisboa 27, Apdo. Postal 6-641, Col. Juarez, Deleg. Cuaubtemoc, 06600, Mexico, DF, Mexico.

*Present addresses: *Regional Research Station, Punjab Agricultural University, Gurdaspur 141 004, India.*

***Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012, India.*

ABSTRACT

The germinating teliospores of *Neovossia indica* produced primary sporidia on water agar and potato dextrose agar, which on germination produced both allantoid and filiform secondary sporidia. The PDA cultures producing both types of secondary sporidia produced, on transfer to liquid medium, either colonies (yellowish granular, settled at the bottom, and whitish leathery, floating on the surface) with clear medium, or a turbid suspension. The liquid cultures had only filiform secondary sporidia. Reinoculation of PDA plates with colonies or suspension resulted in the production of allantoid secondary sporidia. Modification of cultural conditions can lead to the production of one type of secondary sporidia from the other. Conditions with free moisture favoured the production of filiform sporidia whereas conditions with restricted moisture produced allantoid sporidia. Only the allantoid secondary sporidia presumably possess the mechanism to get airborne, and cause infection in nature. The production and collection of fresh allantoid secondary sporidia in large quantities for boot inoculation have been discussed.

INTRODUCTION

THE teliospores of *Neovossia indica* (Mitra) Mundkur germinate by producing a stout promycelium with a whorl of sickle-shaped, long and slender primary sporidia at the tip[1]. The primary sporidia germinate and produce two types of secondary sporidia: (i) falcate or allantoid, and (ii) filiform, similar to primary sporidia in appearance. Warham[2] reported the production of filiform secondary sporidia in liquid cultures, which produced low levels of infection when used for inoculation.

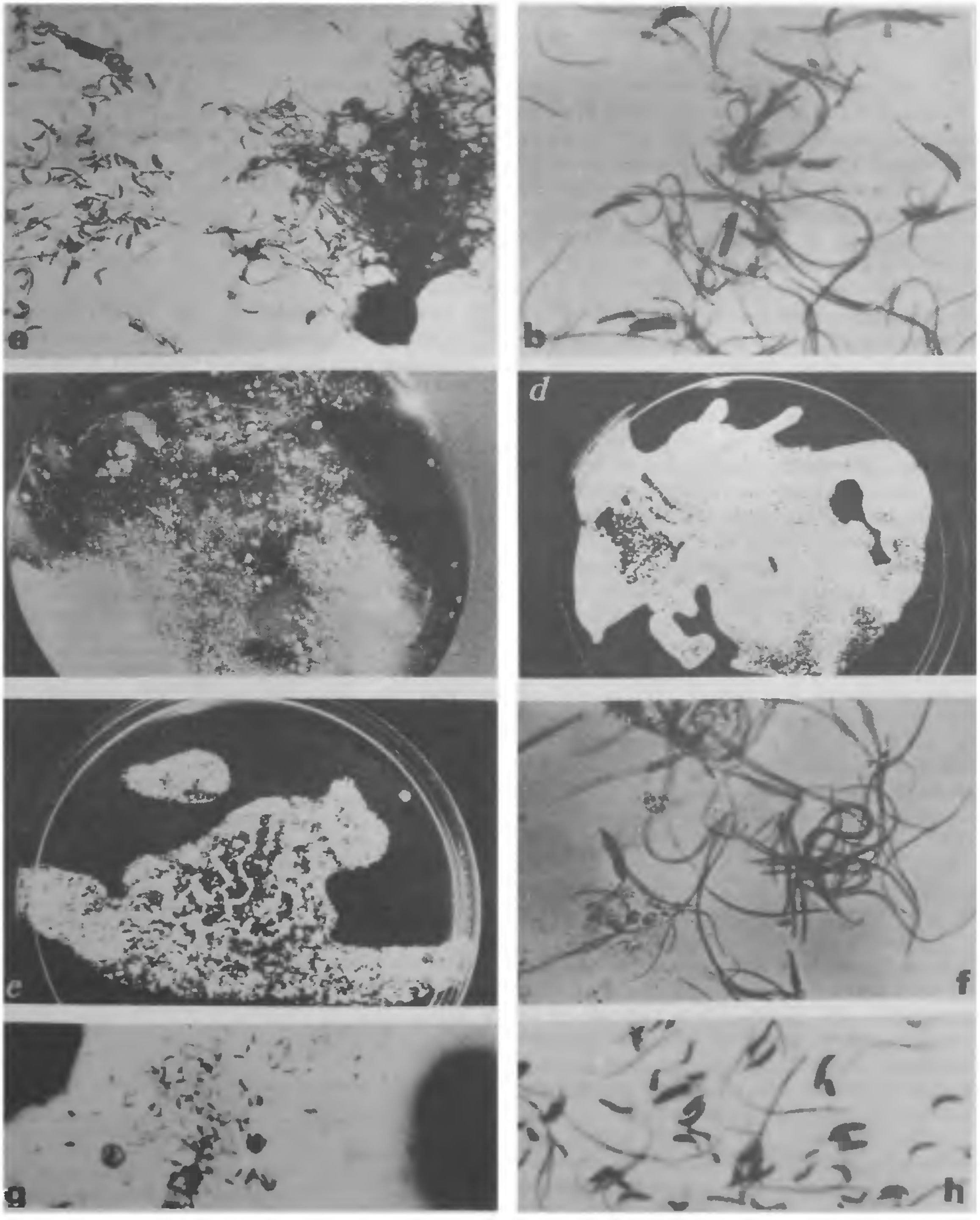
The conditions governing the production of either type of secondary sporidia are not fully known. As a result of several trials for the growth of this pathogen in liquid and semi-liquid media and also on soil substrate, it has become possible to elucidate the particular growth requirements for production of one or both types of sporidia. This study has also been able to pinpoint certain methods or conditions through which the filiform sporidia could be induced to produce allantoid sporidia.

MATERIALS AND METHODS

The teliospores of *N. indica* from bunted grains of 1986 harvest were germinated on water agar using the method described by Warham[2]. The cultures of the pathogen were established on potato dextrose agar (PDA) in petri dishes. After 7 days of incubation at 20°C, 10 ml of sterile distilled water was added to each of the PDA dishes with *N. indica* colonies, the mycelium was gently scraped, and 1 ml of the suspension, containing mainly

†For correspondence.

Figure 1a–h. Production of allantoid and filiform sporidia. **a,** Germinating teliospore on water agar producing allantoid and filiform secondary sporidia. **b,** Allantoid and filiform secondary sporidia produced by germinating teliospore. **c,** Clear suspension with granular colonies at the bottom of liquid culture flask. **d,** Cultures from granular colonies producing allantoid secondary sporidia. **e,** Colonies from clear suspension producing allantoid secondary sporidia. **f,** Freshly inoculated soil producing filiform secondary sporidia. **g,** Colonies on soil producing allantoid secondary sporidia. **h,** Four-week-old colonies on PDA producing both allantoid and filiform sporidia.



Figures 1a–h.

allantoid sporidia, was transferred to 500 ml conical flasks containing potato dextrose broth. The flasks were incubated for one week on a reciprocating shaker.

The two types of colonies produced in liquid culture and the turbid liquid suspension from the flasks were pipetted out separately and plated on PDA dishes. The inoculated dishes were incubated for 2–7 days at 20°C and the production of sporidia was observed. For comparison sporidial production in four-week-old *N. indica* colonies on PDA was observed in a similar way.

Soil from a wheat field of CIMMYT farm at Cd. Obregon (Mexico) was sterilized in petri dishes in a microwave oven for 10 min. The soil was then soaked with sterilized distilled water just enough to wet it. Two or three 1-cm-diameter discs of actively growing cultures of *N. indica* on PDA were stuck to the lids of the petri dishes. The germination and production of secondary sporidia and the formation of *N. indica* colonies on the soil surface were monitored by picking up the sporidia on the sticky side of transparent adhesive tape at one-day intervals.

To identify the type of sporidia released, four-week-old *N. indica* cultures on PDA, producing both allantoid and filiform sporidia, were used. An *N. indica* culture from one petri dish was cut into small pieces of 1 cm^2 and stuck to the lids of petri dishes containing filter papers soaked with the fungicide Tilt (propiconazol). Tilt was used to inhibit the germination of released sporidia and allow unambiguous identification and count. Sporidial discharge was monitored by periodic sporidial counts using a haemocytometer throughout the experiment.

RESULTS AND DISCUSSION

Primary sporidia from the germinating teliospores on water agar mostly remained in the whorl at the promycelial tips. Hyphae from the tuft of the primary sporidia grew very long and produced numerous allantoid, filiform, or both types of secondary sporidia before even the colonies were visible to the naked eye (figure 1a, b).

In liquid cultures two types of colonies were produced in the flasks after 4–5 days of incubation: (i) whitish leathery types floating on the surface, and (ii) small, granular and yellow-coloured types settled at the bottom (figure 1c). Initially the clear liquid suspension had a few filiform secondary sporidia, but when the suspension turned turbid after about 7 days, it had a very high density of filiform sporidia. The results support the observations of Warham[2] that liquid cultures of *N. indica* produce only filiform secondary sporidia. Both types of colonies from liquid cultures, i.e. granular and leathery, and the relatively clear and turbid suspensions, on plating separately on PDA, produced a large number of allantoid sporidia within 24 h of incubation (figures 1d, e). This indicates that the filiform sporidia (from liquid culture) are capable of giving rise to allantoid sporidia when multiplied on solid medium, and vice versa.

Freshly inoculated soil with higher moisture content showed mostly filiform secondary sporidia without much mycelial growth (figure 1f). A few days after inoculation, when the soil moisture content was decreased, a few whitish colonies appeared on the surface. Microscopic examination revealed that the colonies produced allantoid sporidia (figure 1g) in abundance. The results of PDA vs liquid cultures and soil inoculations with allantoid sporidia show that under free moisture conditions filiform secondary.

Table 1 *Discharge of secondary sporidia by mycelium of Neovossia indica on PDA from one petri dish* distributed on the inner surface of seven petri dishes on different days*

Day(s) after start of incubation	Type and total number of secondary sporidia	
	Allantoid	Filiform
1	4,852,600	0
2	7,268,000	0
3	27,924,000	0
4	10,350,000	0
6	5,175,000	0
8**	2,048,000	0
Total	57,617,600	0

*The other petri dishes of the same batch yielded, upon scraping and washing the culture surface, on an average (over five dishes), 226,720 allantoid and 347,360 filiform secondary sporidia; **The experiment was discontinued because of contamination with mycelial pieces due to frequent exposures.

Table 2 *Discharge of sporidia from germinating teliospores of N. indica*

Tilt conc. on filter papers (%)	Type and number of sporidia discharged	
	Allantoid secondary	Primary and filiform secondary
0.1	42,000	0
0.5	48,000	0

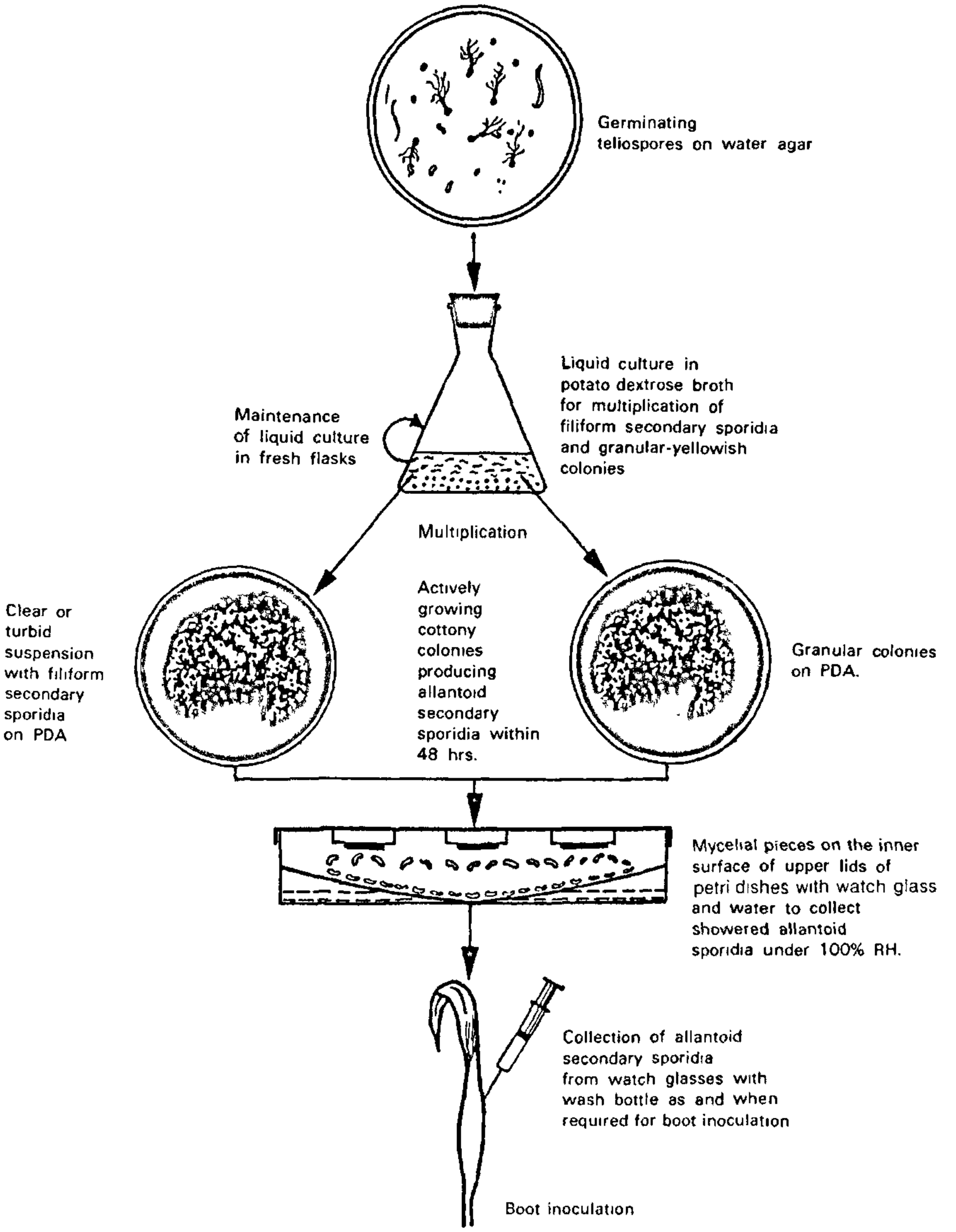


Figure 2. Schematic diagram showing various steps of production and collection of fresh allantoid secondary sporidia in abundance for boot inoculation.

sporidia are produced whereas limited moisture conditions favour production of allantoid sporidia.

The type and number of secondary sporidia discharged from four-week-old mycelial cultures are given in table 1. The discharge of allantoid sporidia occurred within a few hours of the start of the experiment. The four-week-old cultures from the dish, when subdivided and dispensed in seven dishes, discharged more than 57 million allantoid sporidia (table 1). No filiform secondary sporidia was discharged. The allantoid sporidia were released continuously during the day and night for several days. In spore trap studies (Dhaliwal, unpublished) in field conditions also, in Yaqui Valley, Sonora (Mexico), only allantoid sporidia, not filiform secondary sporidia or primary sporidia, were trapped. This observation suggests that filiform sporidia might not have a major role, as the allantoid sporidia, in causing new infection of Karnal bunt in the field. Extremely low incidence of *N. indica* in numerous boot inoculation studies that used high concentration of filiform secondary sporidia[2] also support this conclusion.

Freshly germinating teliospores on water agar, producing primary as well as allantoid and filiform secondary sporidia, also discharged only allantoid sporidia (table 2), further confirming that only the allantoid sporidia are released. The non-release of filiform sporidia in *in vivo* and *in vitro* studies, their exclusive production under free moisture conditions, and rapid switch-over to the production of allantoid sporidia under limited moisture conditions suggest that filiform sporidia might have an important adaptive role in the life cycle of *N. indica* under unfavourable conditions.

The present work opens up the possibility of production of abundant inoculum containing only allantoid secondary sporidia, which can be conveniently collected at intervals of 3–6 h for boot inoculation (figure 2). Cultures obtained from germinating teliospores on water agar can be easily and rapidly multiplied and maintained in potato dextrose broth on shakers. When needed for inoculation, the colonies and turbid medium from such liquid cultures can be plated on PDA in petri dishes. After 3–4 days, inoculum pieces of suitable size from the PDA dishes can be stuck on the inside of lids of petri dishes containing a watch glass and a little water to provide a moisture-saturated environment. Freshly released allantoid secondary sporidia can thus be collected for immediate field inoculations. Before inoculation, the required sporidial concentration (10^4 sporidia/ml) can be obtained by dilution.

ACKNOWLEDGEMENTS

The authors thank Dr M. M. Payak, former Head, Division of Mycology and Plant Pathology, IARI, New Delhi, and Dr S. Nagarajan, Assistant Director-General (Plant Protection), ICAR, New Delhi, for going through the manuscript critically.

11 January 1988; Revised 14 October 1988

1. Mundkur, B. B., *Fungi and Plant Disease*, MacMillan, London, 1949.
2. Warham, E. J., Ph.D. thesis, University of Wales, UK, 1987.