severity of the disease is slightly higher in the dry season.

The leaf spots appear at first as small, oval, dark brown spots, usually towards the leaf tip, later enlarging and becoming pale buff in the centre with a broad, dark brown margin. With multiple infection the lesions coalesce and the leaf becomes blighted from tip to backwards (figure 1). A fungus was isolated from lesions and it was identified as *Bipolaris incurvata* at the Commonwealth Mycological Institute, London (No. 302957). The incidence of the disease was recorded as 0.12% (South Andaman), 0.1% (Little Andaman) and 1% (Campbell Bay) of the palms covered under preliminary survey.

This pathogen was reported earlier as *Helminthosporium incurvata* (Bernad) from countries[1] like Indonesia, Malaysia, Sri Lanka, New Guinea, Virgin Island, French Polynesia, Sahah, Seychelles, New Hebrides and Andaman (India)[2].

The author is thankful to the CMI, London for the identification of the fungus and also to Dr K. K. N. Nambiar, CPCRI for a perusal of the manuscript.

30 November 1987

1. Anonymous, *Nematodes, fungi, insects and mites* associated with coconut palm, Tech. Bull. 2, CPCRI, 1979, p. 60.
2. Anonymous, *Annual Report*, Central Agricultural Research Institute, Port Blair, 1985, p. 54.

UP-TO-DATE LIFE CYCLE OF *NEOVOSSIA INDICA* (MITRA) MUNDKUR

H. S. DHALIWAL* and D. V. SINGH**
International Maize and Wheat Improvement Centre, (CIMMYT), Lisboa 27, Apdo. Postal 6-641, Col. Juarez, Deleg. Cuaubtemoc, 06600, Mexico, D.F.
*Present Address: * Regional Research Station, Punjab Agricultural University, Gurdaspur 143 521, India.*
*** Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012, India.*

Neovossia indica (Mitra) Mundkur, the causal organism of Karnal bunt of wheat was first described from Karnal in Northern India[1]. Since then, Karnal bunt has spread to many countries and frequently assumed epidemic proportions in north-western India, Pakistan and the Yaqui Valley of Mexico[2,3]. There is an urgent need to develop Karnal bunt resistant wheat varieties to avoid deterioration of wheat quality and the spread of the disease to other parts of the world.

An understanding of the life cycle of *N. indica* is useful in the study of breeding for resistance and in controlling the pathogen with chemicals. On the basis of new information collected during the studies in the field and the laboratory at CIMMYT, Mexico, the life cycle of *N. indica* needs to be revised and updated.

Karnal bunt is a seed, soil-borne, floral infecting disease[1,4]. The soil-borne teliospores germinate producing primary sporidia, which are carried by wind to floral structures[5]. The sporidia enter the developing grain either through stigma or through the ovary wall[6–8]. Until recently, little was known of what is occurring in nature during the period between the production of primary sporidia and the appearance of bunted grains in the mature wheat spike.

On potato-dextrose-agar (PDA) medium, the primary sporidia, within the tuft and while still on promycelium, germinate to produce hyphae, which in turn produce two types of secondary sporidia: (i) falcate (allantoid) which are released forcibly[9], and (ii) filiform-like that of primary sporidia which can be easily distinguished from the allantoid form[10,11]. Liquid cultures from the old and newly derived *N. indica* cultures produce only filiform secondary sporidia which when transferred to PDA again, produce allantoid secondary sporidia[10]. Wet soil inoculated with allantoid secondary sporidia produced only filiform sporidia, but on soil under limited moisture had only the allantoid form.

Laboratory studies to study the release of various kinds of sporidia indicated that only the allantoid secondary sporidia were released from the colonies[10]. Spore-trap studies using the Burkard's spore trap under various field conditions trapped only the allantoid secondary sporidia (Dhaliwal, unpublished) between midnight and sunrise. The allantoid sporidia germinate on sterilized leaves of bread wheat, durum wheat, triticale, barley, rye and *Phlaris* and produced superficial colonies which generated more allantoid sporidia (Dhaliwal, unpublished). Warham[11] obtained a low incidence of Karnal bunt from boot and spray inoculation using a high concentration of filiform sporidia from liquid cultures, indicating their limited direct role in the spike infection. Artificial inoculation of the wheat spike with a suspension of secondary sporidia with a hypodermic syringe at the boot stage results in a high incidence of Karnal bunt[12]. The present results indicate that only allantoid secondary sporidia are the real incitant of Karnal bunt in nature and the

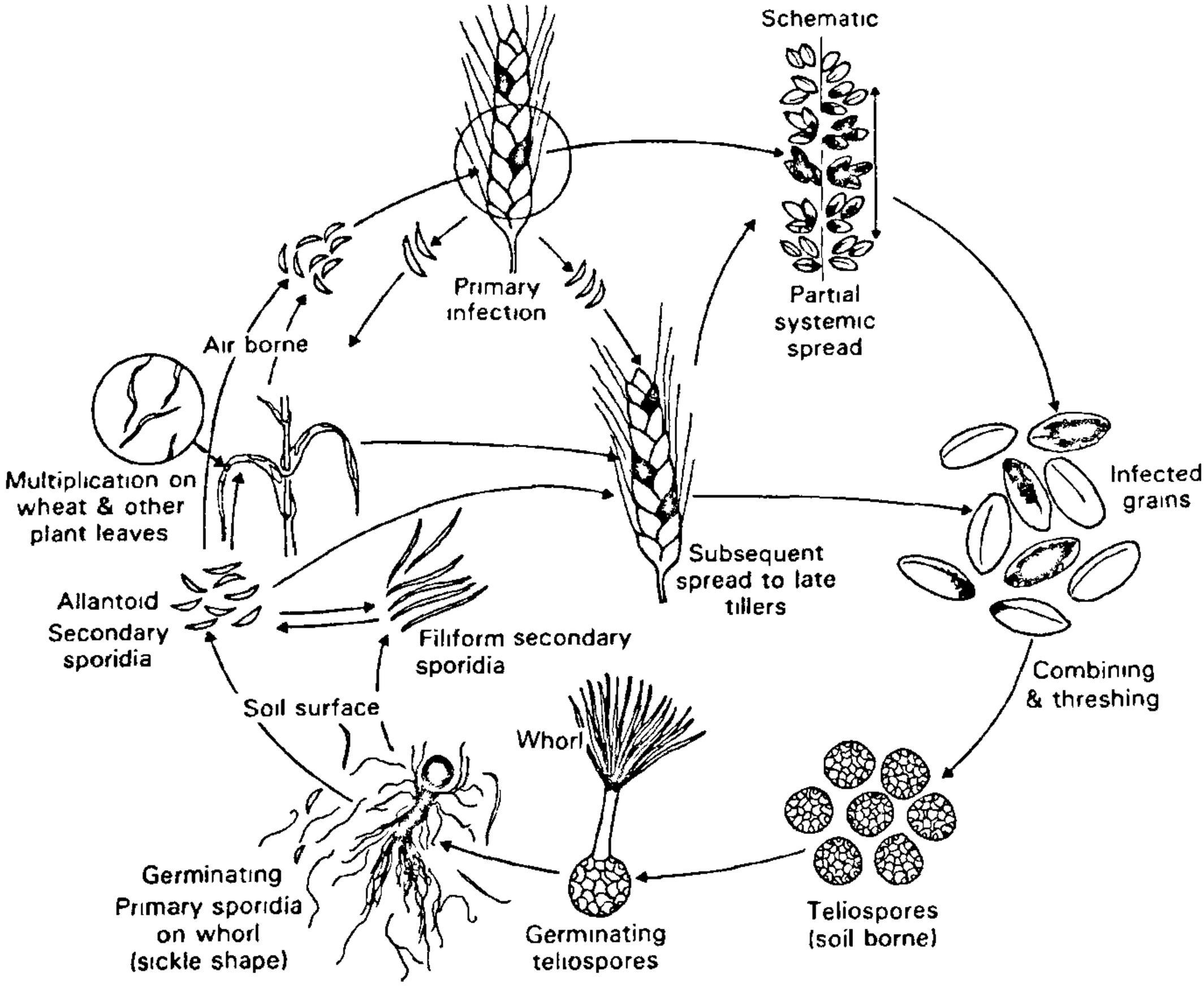

Figure 1. Up-to-date life cycle of *N. indica*.

opening of glumes at anthesis for direct ovary infection is not necessary for the disease to occur. Successful inoculations at boot stage when the glumes are not yet opened to permit the lodging of secondary sporidia on the ovary indicate that the natural infection of Karnal bunt probably also does not take place directly in the ovary during anthesis as widely accepted. The ovaries are probably infected after fertilization, as otherwise the seed set on boot inoculation would be considerably reduced.

The air-borne allantoid secondary sporidia germinate on the glume surface and *N. indica* becomes partially systemic in the rachis and rachilla. Under both natural[13] and artificial inoculation conditions[14]. The disease spreads so adjacent florets and spikelets infecting as many as 31 grains around the primary infection site. The highest damage to the grains occurs in the spikelet at the primary infection site, while the grains with point infection are the result of systemic spread away from infection site.

In the infected spikes, *N. indica* colonies are also established in the florets, especially between glume and lemma or lemma and the dorsal seed surface to produce more allantoid secondary sporidia which are released in the air (Bains and Dhaliwal, unpublished) and are capable of infecting late tillers of wheat (Dhaliwal, unpublished). On the basis of the above information, the revised and up-to-date life cycle of *N. indica* is shown in figure 1.

There are still a few unknown steps in the life cycle of *N. indica*, such as the stage of dikaryotic hyphae formation and the mode of establishement in the rachis. With a complete knowledge of the life cycle of *N. indica*, it will be possible to breed wheat varieties resistant to Karnal bunt or control the disease by more effective use of chemicals.

11 January 1988

1. Mitra, M., *Ann. Appl. Biol.*, 1931, **18**, 178.
2. Singh, D. V., Joshi, L. M. and Srivastava, K. D., In: *Recent advances in plant pathology*,

(eds) A. Hussain, K. Singh, B. P. Singh and V. P. Agnihotri, India Print House, Lucknow, 1983, p. 121.
3. Singh, D. V., In: *Problem and progress of wheat pathology in south Asia*, (eds) L. M. Joshi, D. V. Singh and K. D. Srivastava, Malhotra Publishing House, New Delhi, 1986, p. 124.
4. Mundkur, B. B., *Curr. Sci.*, 1943, **12**, 230.
5. Holton, C. S., *Indian Phytopathol.*, 1949, **2**, 1.
6. Joshi, L. M., Singh, D. V., Srivastava, K. D. and Wilcoxson, R. D., *Bot. Rev.*, 1983, **49**, 309.
7. Munjal, R. L. and Chatrath, M. S., *J. Nucl. Agri. Biol.*, 1976, **5**, 40.
8. Warham, E. J., *Trop. Pest Management*, 1986, **32**, 229.
9. Krishna, A. and Singh, R. A., *Indian Phytopathol.*, 1983, **36**, 115.
10. Dhaliwal, H. S. and Singh, D. V., *Curr. Sci.*, 1988, **57**, (in press).
11. Warham, E. J., Ph.D. thesis, Univ. of Wales, Aberyswyth, 1987.
12. Aujla, S. S., Grewal, A. S., Gill, K. S. and Sharma, I., *Crop Improvement*, 1980, **7**, 145.
13. Dhaliwal, H. S., Randhwa, A. S., Chand, K. and Singh, D., *Indian J. Agric. Sci.*, 1983, **53**, 239.
14. Singh, D. V. and Dhaliwal, H. S., *Seed Res.*, 1988, (in press).

INDUCTION OF ADVENTITIOUS BUDS AND PLANTLET REGENERATION IN *PINUS SYLVESTRIS* L.

S. MOHAN JAIN, R. J. NEWTON and E. J. SOLTES

Department of Forest Science and the Texas Agricultural Experiment Station, Texas A & M University System, College Station, TX 77843-2135, USA.

THE application of tissue culture in forestry is still in its infancy, and it provides an alternative means for cloning trees. Furthermore, it can be used to genetically improve trees over a much shorter time than with traditional technology. Direct induction of adventitious buds on the primary explants without formation of callus, will reduce the risk of chromosomal variation due to cultural conditions. This approach may be applied for clonal propagation in forest trees. By using tissue culture, adventitious buds have successfully been initiated in several pine species[1,5].

In this study, our objective was to induce adventitious buds from embryos, develop shoots and regenerate plantlets.

Seeds of *Pinus sylvestris* L. were stored at 5°C for 2 months. They were sterilized in 10% sodium hypochlorite solution for 15 min and washed in sterile water 4 times. Seeds were placed on a wet filter paper in a petri dish for 3–4 days in the dark. Whole mature embryos were isolated under a binocular microscope and planted horizontally on an agar medium in petri dishes.

Several media were tested for the induction of adventitious buds — Schenk and Hildebrandt[6] (SH) and Gupta and Durzan[7] (DCR). The pH of all the media was adjusted to 5.7 with 1N NaOH or 1N HCl and then autoclaved for 15 min.

Isolated mature embryos were cultured in 100 × 20 mm petri dishes (10 embryos per dish) containing 20 ml of medium. The plates were sealed with parafilm and incubated at 25 ± 1°C, under 2,000 lux light intensity. Induction of adventitious buds directly from embryos was achieved on SH and DCR media supplemented with BAP (0–5 mg/l). All observations were recorded after 4 weeks.

After 4–5 weeks, adventitious buds (1–4 mm) were transferred individually to SH medium containing BAP (0–2 mg/l) for the production of shoots. Shoots (5–10 mm in height) were then removed and cultured on SH medium supplemented with 0.1 mg/l NAA for root induction.

Induction of adventitious buds: Two different media — SH and DCR — were tested for the induction of adventitious buds directly from embryos and development of shoots. Embryos began germination after 72–96 h. The basal end of the embryos turned reddish after 4–5 days. Adventitious buds were formed from the cotyledons of

Table 1 *Effect of medium and BAP on induction of adventitious buds in mature embryos of Pinus sylvestris after 4 weeks*

Medium	Conc. of BAP (mg/l)	No. of mature embryos cultured	No. of mature embryos which formed buds	Per cent
SH	0	42	0	0
	1	40	16	40
	2	40	12	30
	3	40	18	45
	5	38	8	21
DCR	0	40	0	0
	1	40	10	25
	2	40	8	20
	3	40	13	33
	5	40	4	10