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# Production of *Neovossia indica* sporidia on host and non-host plants

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**Abstract**

Inoculated spikes of Karnal bunt resistant triticale and durums developed secondary sporidia of *N. indica* in counts comparable to those from Karnal bunt susceptible bread wheat cultivars. Inoculated spikes of barley, a non-host crop, also produced secondary sporidia. Intact/detached leaves of several crops and weed plants inoculated with *N. indica* sporidia supported the mycelial growth and sporulation of this pathogen. Uninoculated leaves of wheat and five weeds obtained from wheat-fields developed sporidia when incubated in moist saturated atmosphere in the laboratory. *N. indica* appeared to have two distinct phases in its life cycle. The relative significance of these phases in *N. indica*-life is discussed.

## Introduction

Karnal bunt due to *Neovossia indica* (Mitra) Mundkur was first reported on wheat near Karnal in Punjab (now in Haryana) in India (Mitra, 1931). Later, it was reported from other areas also and, at present, it occurs in many countries of the world. Recently, Warham (1986) reviewed the published literature on different aspects of this disease and its pathogen.

Mode of infection of wheat-spikes formed the subject of earlier investigations (Bedi *et al.*, 1949; Mundkur, 1943), where it was concluded that sporidia resulting from teliospore-germination in the soil produced bunted kernels when lodged on spikes. No alternative source of sporidial production in nature was known till Bains and Dhaliwal (1989a;b) reported the production and release of sporidia from infected wheat-spikes. Further investigations on this aspect of *N. indica* revealed that it grew and developed sporidia on resistant and non-host plants also. Such plants appeared to trap sporidia in nature and release the subsequent sporidial crops. Data supporting these observations are presented and, in addition, the occurrence of two distinct phases in *N. indica* life are identified. The relative role of these phases in *N. indica* disease and life cycles are discussed.

## Materials and methods

Experiments were conducted at Punjab Agricultural University, Regional Research Station, Gurdaspur during 1987 to 1989, using plants of the following crops and weeds: *Triticum aestivum* L. cultivars WL 711, PBW 120, PBW 138, PBW 154, HD 2329, C 306; *T. durum* Desf. cv. PBW 34 and Line 921; Triticale cv. TL 1210 and Lines 2167, 2176 and 2188; *Hordeum vulgare* L. cv. Betzes; *Avena sativa* cv. 'Local'; *Pisum sativum* L. cv. Bonneville; *Brassica juncea* Coss. cv. RLM 619; *B. napus* L. cv. 'Local', *Zea mays* L. cv. Pratap; *Cicer arietinum* L. cv. G 543; *Chenopodium album* L.; *Spinacea oleracea* L., *Phalaris minor* L. and *Medicago denticulata* Willd. Plants of different crops and weeds were raised under field or glasshouse conditions. In the field different crops were grown in 4-m, two-row plots. In the glasshouse, plants were sown in earthen pots filled with clay-loam soil.

The sowings were done in November through December of either year. Self-sown weeds in the fields and in pots in the glasshouse were used.

Inoculum consisted of *N. indica* sporidia harvested from cultures raised on potato dextrose agar (PDA) at 20°C for eight to ten days. Inoculation of spikes was done with the syringe method (Chona *et al.*, 1961), as used by Bains and Dhaliwal (1989a). The intact leaves of different crops and weeds were inoculated by spraying sporidial suspension ($1 \times 10^5$ sporidia $mL^{-1}$) in water, using the hand operated sprayer. The spraying was stopped just before the 'run-off'-stage. The detached leaves of different plants were inoculated by showering sporidia from *N. indica* culture, as detailed by Bains and Dhaliwal (1989a). Inoculated spikes developed undisturbed till detached for use in sporidial production. Inoculated, intact leaves were covered with polythene bags for 24 hours after inoculation and, thereafter, exposed to natural conditions (temperature: 16–20°C). Detached leaves were supported on damp cotton in petriplates in the laboratory (temperature: 16–20°C) for the subsequent periods till observations were recorded.

Sporidia released from inoculated spikes were trapped on glass slides with the method of Bains and Dhaliwal (1989b). Leaves replaced spikes in this method where sporidial release from leaves was studied. Sporidia trapped on glass slides exposed to spikes and leaves for specified periods were counted under $10\times$ magnification of a light microscope.

The detached inoculated leaves were examined for sporidial germination and mycelial growth/sporulation 24 and 96 hours after inoculation, respectively. Cello-tape strips mounted on microscope slides (sticky side upward) were gently pressed against the inoculated leaf surfaces. The sporidia and mycelia stuck thereon were examined under the microscope, after adding a drop of lactophenol-aniline blue and a cover glass. In addition, the epidermal peels of the inoculated leaves were similarly stained and examined.

## Results

### *Release of sporidia from inoculated tissues*

*Karnal bunt resistant cultivars/lines.* Spikes of Karnal bunt resistant cultivars and lines of wheat were incubated for sporidial production, 8 to 10 days after inoculation keeping Karnal bunt susceptible cultivars as a check for comparison. Slides exposed to the spikes were examined for sporidial release 24 and 96 hours thereafter.

Slides in all experiments had sporidia. Counts of sporidia from different cultivars and lines overlapped with no appreciable differences within resistant and susceptible types (Table 1).

*Table 1.* Sporidia of *Neovossia indica* trapped under the inoculated wheat-spikes incubated in petri plates in the laboratory

| Species | Cultivar/Line | Reaction to teliospore formation in kernels[a] | Sporidia trapped on slides[b] (No.) |
|---|---|---|---|
| Triticale | Line 2176 | R | 49.0 (37–61) |
| | Line 2188 | R | 60.3 (42–71) |
| | Line 2167 | R | 82.3 (50–107) |
| | TL 1210 | R | 54.3 (47–67) |
| *T. durum* | PBW 34 | R | 50.0 (45–55) |
| | Line 921 | R | 56.0 (57–61) |
| *T. aestivum* | PBW 120 | S | 66.3 (53–75) |
| | PBW 138 | S | 64.3 (43–71) |
| | PBW 154 | S | 62.7 (51–70) |
| | HD 2329 | S | 64.1 (66–72) |
| | C 306 | S | 55.3 (42–65) |
| | WL 711 | S | 62.0 (51–70) |

[a] R = resistant; S = susceptible.

[b] Per $mm^2$ area of the slide with the method of Bains and Dhaliwal (1989b). Figures in parentheses represent range of trapped sporidia.

*Non-host (barley) spikes.* Having discovered sporidial production in Karnal bunt resistant cultivars/lines, it was considered worthwhile to investigate the role of non-host spikes in this phenomenon. Barley cv. Betzes was used, keeping Karnal bunt susceptible wheat cv. WL 711 as a check, for comparison. Spikes of both the crops were inoculated at the boot leaf stage, and detached for incubation 14 days following inoculation.

A total of six independent experiments were conducted during 1988 and 1989. The results of one experiment, where sporidia were trapped for five consecutive days under the same spikes revealed that sporidia were always released irrespective of the crop. Comparatively more sporidia developed in wheat (100 to 150, Average: 117.3) than in barley (77 to 102, Average: 90.9) in this experiment.

*Leaves of different crops.* Leaves of potted plants of different crops (Table 2) were inoculated by spraying sporidial suspension of *N. indica*. Inoculated plants were treated as detailed under Material and methods.

Examination of slides exposed to the detached leaves revealed sporidia in all experiments. The counts of sporidia varied between 50 and 250 per $mm^2$ area of the slides. The same leaves of different crops produced sporidia for 4 or 7 consecutive days (Table 2). Comparison between crops were not made since the leaf areas exposed to slides varied with crops due to differences in leaf-sizes of the crops.

*Table 2.* Release of *Neovossia indica* sporidia from inoculated leaves of different crops

| Crop | Sporidia[a]/Year | |
|---|---|---|
| | 1988 | 1989 |
| *Triticum aestivum* | +(5) | +(7) |
| *Avena sativa* | +(5) | +(5) |
| *Brassica juncea/napus* | +(4) | +(5) |
| *Cicer arietinum* | +(4) | +(4) |
| *Zea mays* | +(5) | +(5) |
| *Hordeum vulgare* | +(5) | +(5) |
| *Pisum sativum* | +(5) | +(5) |

[a] Leaves inoculated by spraying sporidial suspension were detached 10 to 15 days, thereafter and incubated in petri plates. Sign + indicates the presence of 10 or more sporidia per 10× field of a microscope. Figures represent days for which the same leaves were consecutively used.

*Release of sporidia from uninoculated leaves collected from the fields*

*Wheat leaves.* Uninoculated flag leaves of wheat cv. WL 711 in fields having Karnal bunt infected spikes during 1986 to 1987 were collected and incubated for sporidial release during March, 1988. All experiments revealed that such leaves supported *N. indica*. For instance, 50, 80 and 116 sporidia per $mm^2$ area of the slides were trapped in the three independent experiments conducted during 1988.

*Leaves of weed plants.* Uppermost leaves of plants of four different weeds (*C. album*, *S. oleracea*, *P. minor*, *M. denticulata*) were collected from the field mentioned above and incubated in petri plates for sporidial production and release. Presence or absence of sporidia on the slides exposed to the leaves was considered in the first experiment. The observations established that sporidia were released from all the weeds. In the second experiment, where trapped sporidia were counted, respectively, 87, 79, 93 and 103 sporidia were present per $mm^2$ area of slides exposed to *C. album*, *S. oleracea*, *P. minor* and *M. denticulata* leaves.

*Behaviour of* N. indica *on leaves of different crops*

In a separate experiment, where sporidia were showered from *N. indica* cultures on leaves of different crops or weeds and on spikes of wheat and barley, the behaviour of *N. indica* was studied. The inoculated plants were incubated in petriplates under humid conditions. The sporidia/mycelia were taken from them on sticky tapes for observations, specified period after inoculation. Alternatively, the epidermal peels of leaves of different plants were examined. It was found that the sporidia germinated (95 to 100%) on all the test plants. Each germinated sporidium supported two or more germ tubes which elongated and developed into branched, thin mycelia (Plate 1A), resulting into a hyphal net closely appressed to the plant-surface and bearing sporidia. Long stripes of the hyphal-net could be separated from the inoculated plant-surface with the aid of a cellotape or from epidermal strips with the gentle touch of a brush.

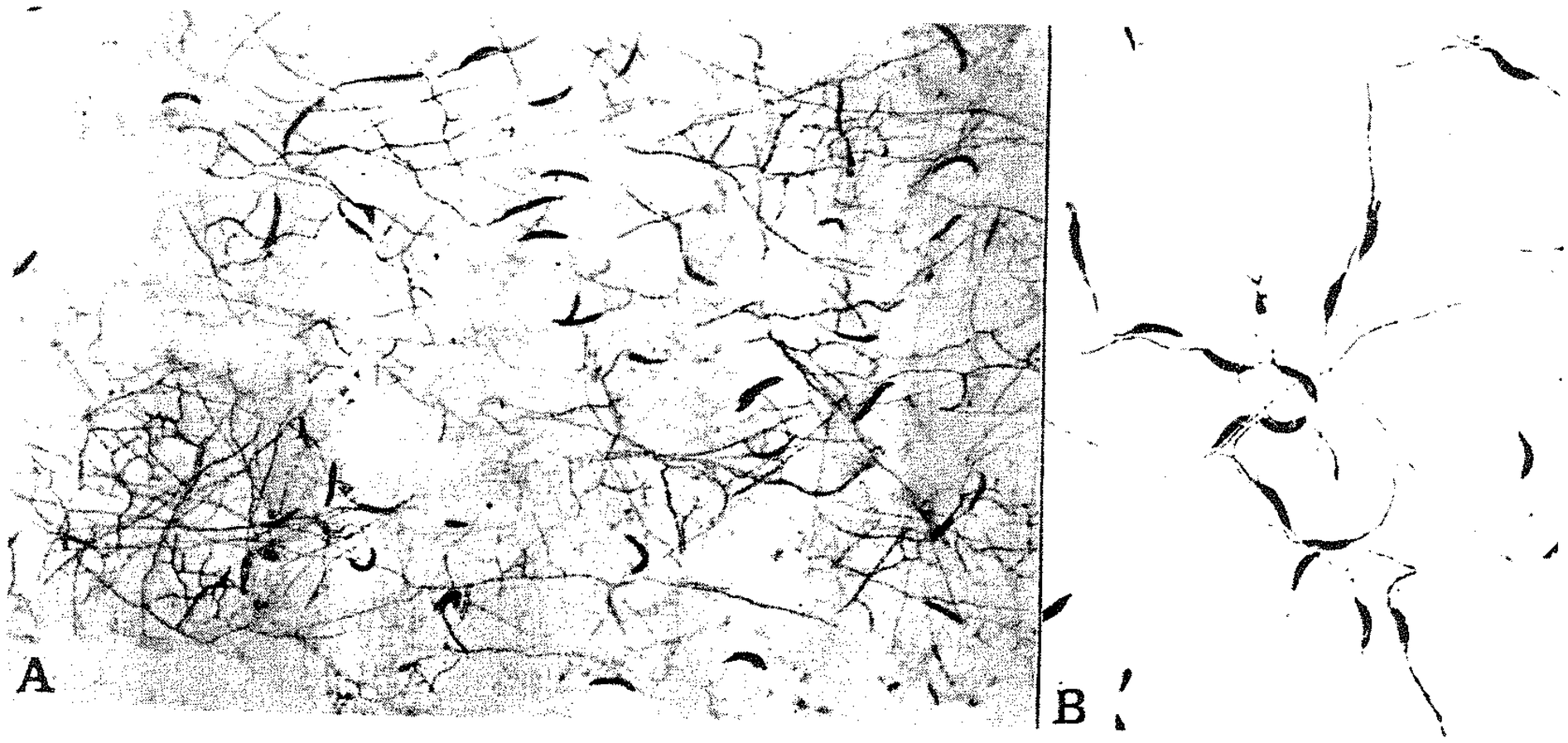


*Plate 1. Neovossia indica* growth on wheat tissue (**A**) and germination of sporidia on a glass slide (**B**)

*Nature and viability of trapped sporidia*

Exclusively allantoid sporidia were released, irrespective of the crop, weed or tissue (spikes/leaves), of plants used. Normal sporidia developed on green leaves. Very small sporidia developed on detached senescent leaves of different plants. Normal sporidia trapped from all sources germinated (Plate 1B) when incubated at 20°C on slides supported in petri plates lined with moist filter paper discs. A germinated sporidium had one or more (mostly two) germ tubes some of which produced upto four sporidia after 96 hours of incubation. Such sporidia were smaller than the normal ones.

## Discussion

This investigation proved that inoculated spikes of Karnal bunt resistant wheat non-host barley and leaves of wheat and non-host crops/weeds developed *N. indica* sporidia when incubated under humid conditions. Sporidia trapped from these sources were invariably allantoid-type and appeared identical in morphology and in their *in vitro* germination-behaviour. This investigation also revealed that wheat cvs. supporting fewer or no teliospores in their kernels developed as much sporidia as the susceptible cv. WL 711. Earlier, Bains and Dhaliwal (1989a;b) reported the production of *N. indica* sporidia from spikes of susceptible wheat cv. WL 711.

In a field planted with wheat, plants occupy a major part of the aerial space above the ground/canopy level. Part of the remaining space over such fields may be occupied by the foliage of weed-plants. It was, therefore, logical to conceive that many of the sporidia, released from infected spikes (Bains and Dhaliwal, 1989a) in such fields were trapped on leaves of wheat and weed plants. Likewise, the sporidia originating from teliospore-germination in the soil (Bedi *et al.*, 1949) might also lodge on these plants. The present investigation, where uninoculated leaves of wheat and weed plants collected from nature released sporidia following their incubation under humid conditions in the laboratory supported this hypothesis. In this phenomenon, the sporidia trapped on plant surfaces established *N. indica* growth, giving rise to subsequent sporidial-crops. Observations on the behaviour of *N. indica* on inoculated plants also supported this conclusion.

On glass slides, *N. indica* sporidia produced germtubes forming a few secondary sporidia only. However, *in vivo N. indica* developed a thick mycelial net on different crops and weeds. It, thus, appears that *N. indica* gets nutrient support from the inoculated plants, irrespective of the susceptibility of such plants to *N. indica*. The ease with which the mycelia were separated from the inoculated plants indicates that, apparently, such plants supported the superficial growth of *N. indica*. No additional observations were made to support this conclusion further.

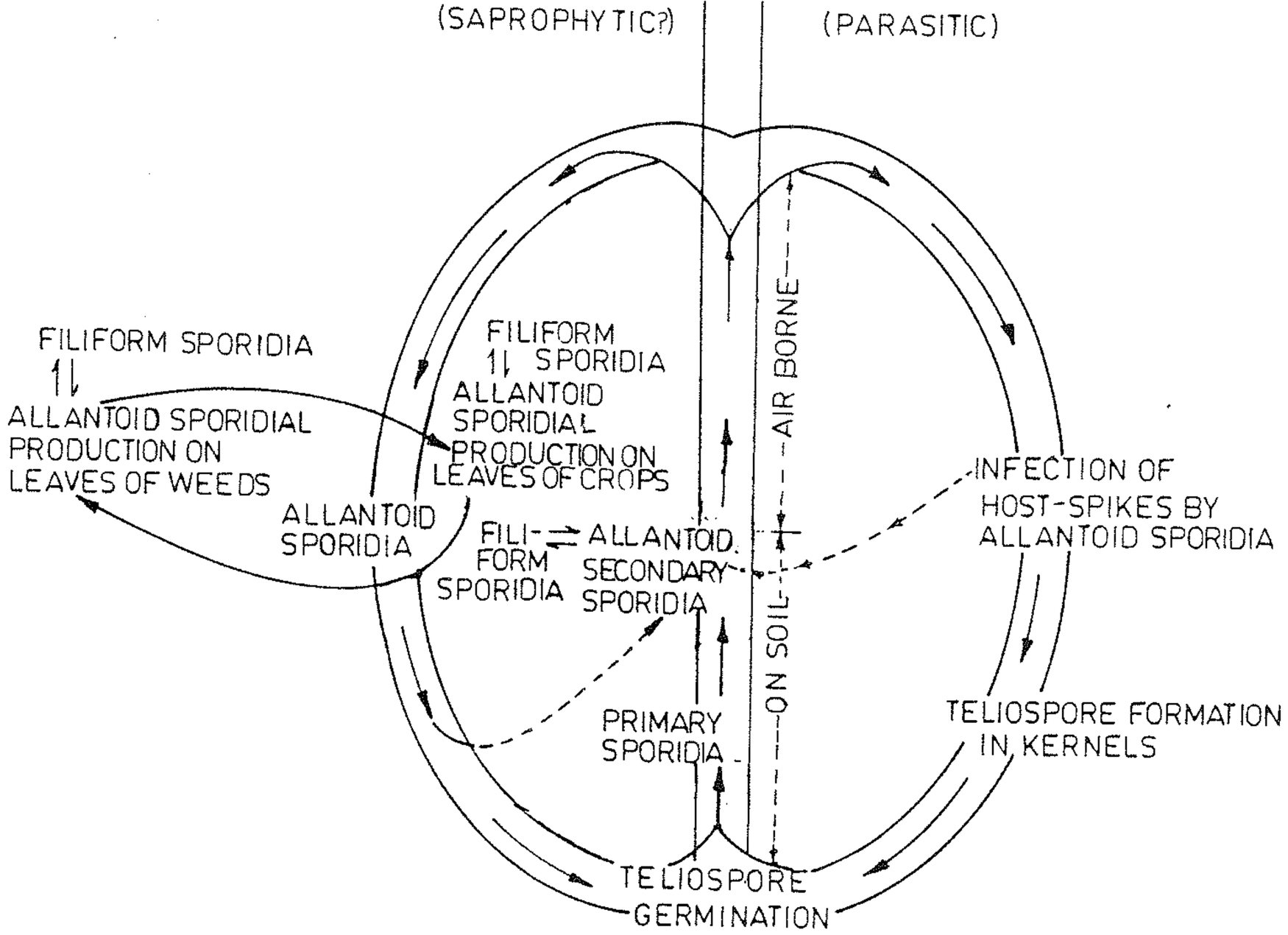


*Fig. 1.* Two distinct phases, *i.e.* sporidial phase and teliospore phase, in the life of *Neovossia indica*

The information published to date (Warham, 1986) reveals that *N. indica* develops teliospores in kernels of its host-plants and this phenomenon, *i.e.* the teliospore formation, is the culmination end of parasitic activity of *N. indica* in a season. In contrast, sporidial production, which needs no specialised tissue or plants, appears to represent saprophytic activity of *N. indica* life. On the basis of these differences and the time-lapse between teliospore to teliospore and sporidia to sporidia cycles (Bains and Dhaliwal, 1989a) the life cycle of *N. indica* appears to have two distinct phases, *i.e.* sporidial phase and teliospore phase (Fig. 1). The two phases appear to play different roles in the life of *N. indica*. The teliospores, being the hard structures and exhibiting dormancy, appear to contribute in the carry over of *N. indica* across the crop seasons. They appear to provide the initial inoculum as a result of their germination in the soil. Following this, the sporidial phase appears to play the main role by producing sporidia through repeated sporidia to sporidia cycles (Fig. 1).

The sporidial phase requiring no specialised hosts or their tissues, appears to contribute to the abundance of inoculum, not expected otherwise in nature. Because of this reason, probably, *N. indica* is able to avail of the combination of availability of spikes in their susceptible phase, the requisite favourable weather factors, and the requisite compatible mating groups to produce successful infections, apparent as bunted kernels in nature.

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