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Release of secondary sporidia of *Neovossia indica* from inoculated wheat spikes

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Abstract

Wheat-spikes inoculated with *N. indica* at the boot-leaf stage produced secondary sporidia when later incubated (intact/detached) under moist conditions in the laboratory. Sporidia were also released from inoculated spikes in the field where sporidial release exhibited diurnal periodicity. More sporidia were trapped between 5–6 o'clock than during the later parts of the day but no sporidia were trapped between 14–18 o'clock. However, they could be trapped at any time of the day from the detached spikes incubated under moist conditions in the laboratory. Sporidia trapped in different experiments were invariably of the allantoid type and they proved viable and infective. Maximum sporidia developed on the outer glumes of florets, and this observation was supported by scanning electron microcope studies. Sporidia developed at 15 and 20° C but not at 30° C. These findings indicated that repeated cycles of sporidial production in spikes provided more inoculum than expected from soil-borne teliospores of *N. indica*.

Introduction

Neovossia indica (Mitra) Mundkur causing Karnal bunt of wheat was first reported from India in 1931 (Mitra, 1931). Several aspects of this pathogen were later investigated (Chona *et al.*, 1961; Dhaliwal *et al.*, 1983; Holton, 1949; Krishna and Singh, 1982; Mitra, 1935, 1937; Warham, 1986) and it was concluded that teliospores developing in kernels became soil-borne directly or through seed. Such teliospores germinated on the soil to produce primary sporidia in the subsequent crop season (Mundkur, 1943; Holton, 1949; Warham, 1986) and from these the secondary sporidia developed and became air-borne (Mundkur, 1943; Bedi *et al.*, 1949). They infected developing wheat kernels after lodging on the spikes (Bedi *et al.*, 1949). In this way, all infections of spikes were considered to result from inocula exclusively produced in the soil (Mundkur, 1943; Bedi *et al.*, 1949).

We observed that *N. indica* produced secondary sporidia from inoculated wheat spikes. Such sporidia were viable and infective, thus having potential for new infections. Data supporting these observations are presented.

Materials and methods

Experiments were conducted for three years (1986 to 1988), using *Triticum aestivum* L. cultivar WL-711. Plants were raised in loamy soil in earthen pots (diameter 30 cm) in the glasshouse and in beds of 4.0 × 2.5 m in the field. Sowings were done in November/December of each year and fertilizers, as recommended for wheat in the Punjab (N : 50, P : 25, K : 25 kg acre^{-1}) were applied.

Inoculum consisted of sporidia harvested from one-week-old cultures of an isolate of *N. indica* raised on potato dextrose agar (PDA) at 20° C (exceptions mentioned in the text). Plants at the boot-leaf stage (stage 10 on Feekes' scale) were inoculated with the aid of a hypodermic syringe (Chona *et al.*, 1961). Each spike received 1 × 10^5 sporidia in mL^{-1} water. Detached spikelets were used in scanning electron microscopic (SEM) stu-

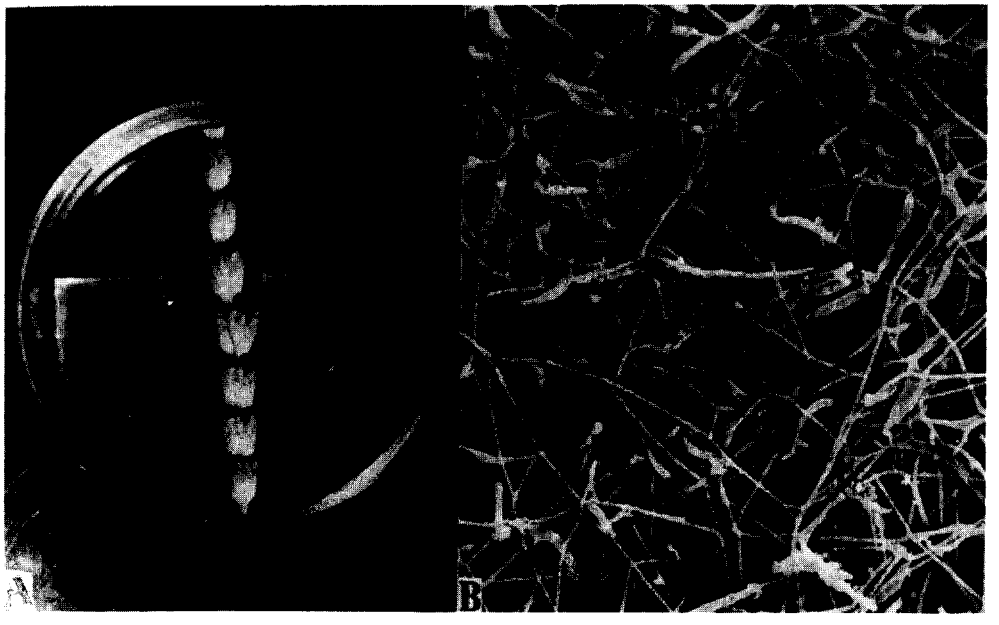

Plate 1. Assembly showing incubation of detached spikes for sporidial production *(A)* and a photomicrograph *(B)* exhibiting mycelial growth and allantoid sporidia on the outer glume of a floret.

dies. Spikelets, sterilized in a 5 per cent solution of commercial Cloralex (6% NaHC10) for 8 to 10 min, were aseptically seeded on sterilized, moist, filter paper discs in the lower halves of petri dishes. The upper lid of each petri dish was seeded with a disc (1 cm^2) of PDA supporting *N. indica* culture. This was held in position so that sporidia showered on the spikelets underneath. This assembly was incubated at 20° C with 12 h photoperiods. The spikelets were processed for SEM studies after specified periods of incubation following inoculation.

Release of sporidia from the inoculated spikes was studied under both laboratory and field conditions. Awns and distal one-thirds of intact spikes were clipped off 8 to 10 days after inoculation. Such samples were held directly against the dry slides in closed, humid chambers. Additionally, whole detached spikes (Plate 1) were used. The slides collected from underneath the spikes (intact/detached) were examined for sporidia under the microscope. The counts were made on samples from at least five different plots (10 ×) manifesting sporidia.

Separated parts of florets (glumes, lemmae, paleae, ovaries) of spikes inoculated at the boot-leaf stage, were used to determine their contributions to sporidial production. Outermost florets, from different spikelets, were detached and separated, from the florets, into different parts. Ten glumes/lemmae/paleae/ovaries, were added to a test tube containing 0.5 mL of distilled water. The tubes were tightly plugged and incubated at specified temperatures in a BOD incubator. After 24 or 48 hours, another 0.5 mL of water was added per test tube; the tubes were hand shaken for 2 min and the eluted sporidia were counted haemacytometrically. Three replicate tubes were used for each part of the florets.

In the field, the vaseline coated 'cello-tape' strips, supported on iron platforms, were exposed to the inoculated spikes held parallel to the platforms with the aid of rubber bands. The tapes were collected at different hours and examined for sporidia. Sporidial counts were made from at least five transverses across the width of a slide.

The *in-situ* germination of teliospores was examined in spikes harvested at different intervals after inoculation. *In-vitro* germination of teliospores and sporidia was tested in sterilized distilled water, and on dry slides incubated under moisture saturated conditions, at 20° C. Observations were recorded after specified periods of incubation.

In vivo sporidial germination and subsequent hyphal development was studied, using SEM model JE, JSM-35. The tissues were fixed in 3 per cent

glutaldehyde in phosphate buffer for 3 hours. These samples were washed in phosphate buffer before and after fixing them in 2 per cent osmium oxide for 2 h. They were then dehydrated by sequential immersion in 30, 60, 70, 80, 90, 96 and 100 per cent ethanol, with the samples remaining for 2 h in each concentration. Then the samples were dried finally with a critical point dryer Samdri-780A using CO_2 and coated with gold in a fine coat ion sputter (Model JFC-1100). The observations were made at different intervals after inoculation.

Results

Release of sporidia from spikes

Examination of slides exposed to intact spikes in the laboratory revealed the presence of sporidia in all the experiments conducted during 1986 to 1988. The results of two experiments (one each in 1986 and 1987) are summarised in Table 1. In both the experiments, fewer sporidia were trapped on slides exposed during the first 24 hour periods. Sporidial counts increased with the subsequent exposure of slides to the spikes (Table 1). Release of sporidia from such spikes continued several weeks after inoculation (maximum period tested three weeks).

Sporidia were also trapped on slides exposed to the inoculated, detached spikes contained in petri dishes. A minimum period of 7 hours was required for the spikes to be in the humid conditions in the petri dishes before the sporidial release started. Sporidia were released, irrespective of the time of the day the spikes were detached.

Table 1. Sporidia[a] of *Neovossia indica* trapped on slides exposed to intact wheat spikes[b] in the laboratory

Days after inoculation	No. of sporidia/year (± S.D.)	
	1986	1987
12	0.1	0.4 ± 0.6
13	14.0 ± 8.5	33.1 ± 12.1
14	13.2 ± 5.5	12.3 ± 3.9
15	11.6 ± 4.6	14.0 ± 3.5
16	6.8 ± 4.3	80 ± 38.0

[a] per mm^2 area of a slide. Figures are based on 5 and 10 independent experiments during 1986 and 1987, respectively.
[b] Inoculated spikes were held in a moisture saturated atmosphere in a closed chamber. Observations were repeated every 24 hours.

Table 2. Sporidia of *Neovossia indica* trapped underneath inoculated spikes at different times under the field conditions

Time of exposure of slides (hours)	Sporidia[a] (No.)
5–6	23.0
6–10	3.3
10–14	0.16
14–18	0.0
18–5 (next day)	6.9

[a] Per 1 mm^2 area of the slide exposed to a spike. The figures are based on five independent experiments.

Sporidia were also found on tapes exposed to the inoculated spikes in the field (Table 2). Tapes collected at different hours revealed periodicity in sporidial release. More sporidia were trapped in the early morning hours; counts of trapped sporidia dropped at the time of the day advanced and no sporidia were trapped between 14 to 18 o'clock (Table 2).

Relative contribution of different floral-parts in sporidial production

Sporidia developed in all floral-parts. However, more sporidia developed in outer glumes, compared with other parts (Table 3). This trend persisted in repeated experiments and with the increase of incubation periods from 24 to 48 hours (Table 3).

Examination of inoculated spikelets under SEM revealed heavy growth of mycelia of *N. indica* on the outer glumes. Sporidia developed in all these cases were of the allantoid type (Plate 1).

Relation of temperature to sporidial production

In a separate set of experiments, production of

Table 3. Production of *Neovossia indica* sporidia in different parts of florets of wheat spikes, inoculated at boot leaf-stage

Parts of the floret	Sporidia/Time of incubation (hours) (1×10^5)	
	24	48
Glume	33.0	69.0
Lemma	9.7	20.0
Palea	3.3	3.3
Ovary	1.0	2.0

Per ten parts in 1 ml of water.

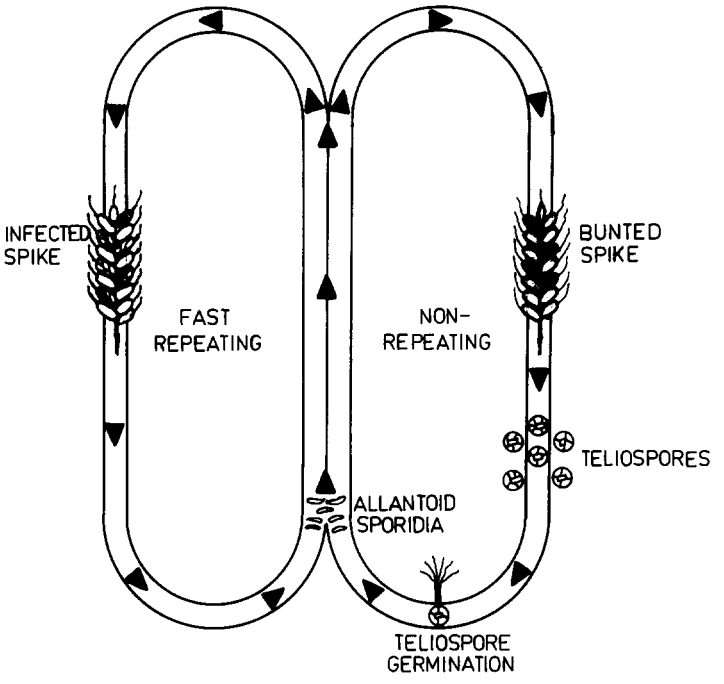

Fig. 1. Diagram showing teliospore germination in the soil to teliospore production and allantoid sporidial formation in the infected spikes. The two halves of this cycle in that order represent 'non-repeating' and 'fast repeating' cycles of sporidial production.

sporidia at 15, 20 and 30° C was examined, using the detached glumes and lemmae. Sporidia were present in glumes and lemmae incubated at 15 and 20° C (Table 4) but they did not develop in these tissues incubated at 30° C. More sporidia developed at 20° C than at 15° C, irrespective of the floral parts used (Table 4).

Nature, viability and infectivity of trapped sporidia

Both allantoid and filiform sporidia developed on PDA in the laboratory. However, sporidia trapped underneath spikes in the laboratory or field experiments were invariably of the allantoid type (Plate 1). The exceptions were experiments where the detached floral parts were closely associated with water. In these tests both allantoid and filiform sporidia were present.

Allantoid sporidia germinated by putting out germ tubes. Between 30.5 to 100.0 per cent sporidia germinated within 4 to 12 hours in different experiments.

Sporidia (collected from underneath the detached spikes) suspended in water and used to inoculate wheat spikes at the boot-leaf stage, resulted in bunted spikes in all the 25 tests during 1986 and 1987.

Table 4. Relation of incubation temperature to sporidial production on detacted parts of florets

Temperature (°C)	Sporidia/part of the floret (1×10^5)	
	Glume	Lemma
15	14.8	3.0
22	91.8	18.8
30	0.0	0.0

Teliospores in inoculated spikes and their relation to sporidial release

Intact spikes, which were earlier used in sporidial trap studies, produced bunted kernels at maturity in the glasshouse. In one experiment, the incidence of bunted kernels ranged from 7.5 to 30.0 per cent in different spikes. Teliospores from these kernels failed to germinate when incubated in water immediately after harvest. Teliospores in bunted kernels did not germinate. This excluded the possibil-

ity of *in situ* germination of teliospores and thus their role in sporidial production.

Discussion

In this investigation, inoculated spikes produced secondary sporidia under field and laboratory conditions. Earlier, it was believed that all inoculum in the field originated from the soil (Bedi *et al.*, 1949).

Spikes used in spore trap studies in this investigation were inoculated several days prior to their use for sporidial production. Such spikes released sporidia continuously for weeks. The released sporidia did not, however, appear to originate from teliospores formed in the kernels of the spikes as teliospores developed several days after the sporidia were trapped. There was no evidence of *in-situ* teliospore germination, and furthermore, the freshly harvested teliospores from the spikes did not germinate in this or in other earlier investigations (Krishna and Singh, 1982; Mathur and Ram, 1963; Mitra, 1935). Therefore, it appeared that the trapped sporidia originated from *N. indica* mycelia in/on the inoculated tissues. SEM studies revealed growth and reproduction of *N. indica* on the glumes. and thus, provided support for the above conclusion.

Sporidia trapped in these studies proved to be of only one type, *i.e.* allantoid-type. Filiform sporidia developed in inoculated tissues kept in contact with free moisture. The factor deciding the type of sporidia to be produced, was thus, the availability of free moisture.

Warham (1987) reported that inoculation of wheat spikes with filiform sporidia resulted in few bunted kernels than when comparable counts of allantoid sporidia were used. This investigation, where the sporidia trapped on slides were always of allantoid type, indicated that probably these structures played an exclusive role in establishing new infections under normal conditions in the field. Filiform sporidia may have a role in this process in the presence of free moisture and the agencies causing their release from the tissues.

Allantoid sporidia exhibited a diurnal periodicity of their release in nature. Sporidia were, however, released irrespective of the time of the day in the laboratory where continuous humid periods were provided. Moisture periods, therefore, appeared associated with the maximum release of sporidia in the early hours of the day.

Allantoid sporidia released from the inoculated spikes proved viable. They resulted in bunted kernels when used to inoculate wheat spikes at the boot-leaf stage. Thus, contrary to the earlier conclusions, where it was believed that all infections in the spikes resulted from sporidia originating from teliospore germination in the soil (Bedi *et al.*, 1949), this investigation points to the possible involvement in fresh infections, of sporidia produced in the infected tissues. Allantoid sporidia appear to have a significant role in this process as they were the sporidia trapped exclusively in different studies.

Teliospores released into the soil germinate in the subsequent season when the wheat crop is at the heading stage (Bedi *et al.*, 1949). Thus, teliospore germination to teliospore formation, after fresh infections in the spikes (Figure 1), combines two crop seasons. However, sporidial production in infected spikes is fast and it completes several cycles within the same season (Figure 1). This part of the life cycle of *N. indica*, thus, contributes much more to the inoculum build-up in nature.

References

Bedi K S, Sikka H R and Mundkur B B 1949 Transmission of wheat bunt due to *Neovossia indica* (Mitra) Mundkur. Indian Phytopath. 2, 20–26.

Chona B L, Munjal R L and Adlakha R L 1961 A method for screening wheat plants for resistance to *Neovossia indica*. Indian Phytopath. 14, 99–101.

Dhaliwal H S, Randhawa A S, Karam Chand and Dalip Singh 1983 Primary infection and further development of Karnal bunt of wheat. Ind. J. agric. Sci. 53, 239–244.

Holton C S 1949 Observations on *Neovossia indica*. Indian Phytopathol. 2, 1–5.

Krishna A and Singh R A 1982 Effect of physical factors and chemicals on the teliospore germination of *Neovossia indica*. Indian Phytopathol. 35, 448–455.

Mathur S C and Ram S 1963 Longevity of chlamydospores of *Neovossia indica* (Mitra) Mundkur. Sci. and Cult. 29, 411–412.

Mitra M 1931 A new bunt of wheat in India. Ann. Appl. Biol., 18, 178–179.

Mitra M 1935 Stinking smut (bunt) of wheat with special reference to *Tilletia indica*. Mitra. Indian J. Agric. Sci. 5, 51–74.

Mitra M 1937 Studies on the stinking smut or bunt of wheat in India. Indian J. Agric. Sci. 7, 469–478.

Mundkur B B 1943 Karnal bunt, an air-borne disease. Curr. Sci. 12, 230–231.

Warham E J 1986 Karnal bunt disease of wheat: A literature review. Tropical Pest Management 32, 229–242.

Warham E J 1987 Studies on Karnal Bunt Disease of Wheat. Ph.D. Thesis submitted to University of Wales, UK.