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Physiological and Morphological Aspects of *Bipolaris sorokiniana* Conidia Surviving on Wheat Straw

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Wheat samples showing typical spot blotch symptoms on stems and sheaths were collected from the field after physiological maturity, and were sealed in paper bags and stored in the laboratory at room temperature to study the survival of *Bipolaris sorokiniana* conidia on wheat straw. The materials were observed at monthly intervals to assess the conidia viability during storage. After 4 months, the frequency of individual conidia already present on wheat straw at the time of sampling was reduced and appeared to be progressively replaced by the formation of round structures consisting of conidia aggregates. After 5 months, distinct, individual conidia were no longer detected, and only 'clumps of conidia' were observed. These dark black aggregates or 'clumps of conidia' measured 157-170 μm in diameter and were grouped into boat-shaped olivaceous conidia showing thick wall and measuring 50-82 × 20-30 μm. The germination was unipolar and below 0.5%, suggesting the occurrence of dormancy. In contrast, individual conidium produced on wheat during the growing season were 96-130 × 16-20 μm, slightly curved, hyaline to light pale, and euseptate with a bipolar germination reaching 98-100%. *Bipolaris sorokiniana* conidia produced on PDA were 55-82 × 20-27 μm, tapered at both ends, dark brown to olivacious, distoseptate, showed up to 1% germination, and were predominantly unipolar. Results of the present study suggest that *B. sorokiniana* conidia belonged to two different physiological categories corresponding to the pathogen's infection phase and its survival, respectively. The infection phase is characterized by a high germination percentage as opposed to the survival phase harboring apparent dormancy.

Keywords **:** *Bipolaris sorokiniana, Cochliobolus sativus*, conidia, dormancy, spot blotch, survival, viability.

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Cochliobolus sativus (Ito & Kurib.) Drechsler ex Dastur (anamorph: *Bipolaris sorokiniana* [Sacc.] Shoem.), a wheat pathogen showing a broad range of alternate hosts, is distributed worldwide and causes substantial grain losses in warmer growing areas in the subtropics (Nelson and Kline, 1962; Duveiller et al., 1998). The fungus induces several types of symptoms on wheat and barley including root rot, leaf blight, or spot blotch (Duveiller and Garcia Altamirano, 2000). Annual yield losses caused by the pathogen vary according to cropping system, sowing date, and locations. Grain losses range from 5% to 85%, but are estimated to reach, on average, 15% annually in South East Asia (Duveiller and Gilchrist, 1994; Saari, 1998; Dubin and Duveiller, 2000).

Bipolaris sorokiniana is an ascomycete characterized by a profuse production of dark conidia that germinate within 5-7 hours in the presence of free water (Misra, 1973). The pathogen survives in soil, plant debris, and on seed (Mehta, 1993; Reis et al., 1995; Duzeck et al., 1996). Since little is known on the survival of *B. sorokiniana* conidia on wheat stubbles after the crop is harvested, this study was undertaken to observe the conidia and their viability on wheat straw stored at room temperature, as a step toward an integrated control approach of foliar blights in wheat.

Materials and Methods

Samples presenting typical spot blotch lesions on different plant parts were collected from various hosts of *B. sorokiniana* including wheat, barley, *Phalaris* spp., and *Setaria* spp. during the month of April 1999, coinciding with the end of the wheat-growing season in eastern Uttar Pradesh, India. Lesions were observed under the compound microscope for the presence of *B. sorokiniana* conidia and morphological features such as shape, size, and color were recorded. *B. sorokiniana* was isolated on Potato Dextrose Agar (PDA) in order to produce monoconidial strains for future studies. These strains were stored at room temperature (25±2°C) on PDA slants.

The germination of conidia collected from the living and dead plant parts of different hosts and from cultures on PDA was tested

using the glass slide technique proposed by Chinn (1953). Conidia were mixed in a drop of sterilized water on a microscope slide placed in a Petri dish containing sterile-water-soaked cotton lining to provide a moist condition and incubated at 28°C for 48 hours. The germination percentage was observed every 4 hours and the type of conidia germination, unipolar or bipolar, was evaluated on a total of 250 conidia.

Seeds of popular wheat cultivars were collected just before harvest and examined for the presence of conidia before storage for 1-5 months (from May to September). The conidia adhering on external seed coats were scraped, mounted on cotton blue lactophenol, and observed under the microscope.

The samples, including the infected parts (leaves, stems) and the seeds showing abundant conidia, were stored at room temperature in 50 separate packets, in which a naphthalene ball was added to deter mycophage mites and insects. The germination percentage and morphology (shape, size, and color) of conidia produced on PDA were also examined.

The pathogenicity of conidia found on living or dead plant parts was tested after inoculating 10^4 conidia/ml on the susceptible genotype HUW234 at flag leaf stage using the method proposed by Duveiller et al. (1998). All inoculated plants were incubated at 25°C for 48 hours in a polyethylene screenhouse equipped with a sprinkler to maintain the wheat leaf surface wet. The incubation period until the first symptoms appeared and the number of lesions per leaf area were recorded.

Results

The size and shape of *B. sorokiniana* conidia produced on the host during the disease development in the field (pathogenic phase) were different from those of the conidia observed on the straw during storage, as well as, from those of conidia produced on PDA (Table 1). Conidia collected directly from the host plants standing in the field were large, euseptate, and measured, on the average, 96-124 × 16-20 µm (Fig. 1a) compared with the relatively smaller size, 55-82 × 16-20 µm, observed on stored dead plant parts or on the culture medium (Fig. 1b). Also, conidia collected during the pathogenic phase were hyaline and showed blue vacuoles when mounted in cotton-blue lactophenol. In contrast, conidia produced on PDA or found on the host during storage appeared dark brown and distoseptate (Fig. 1b).

When the conidia were collected from the living plant parts, the germination was bipolar and reached, on the average, 97.5-99.8%. However, conidia produced on PDA and found on straw during storage were predominantly unipolar and showed 1% germination suggesting a different physiological condition corresponding with the survival or saprophytic growth on artificial culture medium (Table 1).

Conidia directly produced on the host differed in pathogenicity from conidia that developed on PDA or those collected from stored straw (Table 1). Although no difference was observed in the incubation period which reached 5 days between inoculation and first symptom appearance under the study conditions, the average number of lesions per cm^2 (lesion density) was significantly higher (11.5) after inoculation of susceptible wheat plants with conidia obtained directly from the living host, compared with the number of lesions per cm^2 (2.5) resulting from inoculation with conidia collected from stored straw or PDA (Table 1).

After 4 months of storage at room temperature, it became difficult to detect hyaline conidia on stored materials and no single conidia could be observed during the following weeks (Table 2). The formation of conidia aggregates was noticed in the paper bags containing infected straw and sheaths. These aggregates or 'clumps of conidia' suggesting fungal structures morphologically similar to sclerotia,

Table 1. Characteristics of *Bipolaris sorokiniana* conidia produced on the hosts and on PDA

Character	Isolates from				PDA
	Wheat	Barley	*Phalaris* spp.	*Setaria* spp.	
Size (µm)	96-124 × 16-21	75-115 × 13-20	87-100 × 13-21	78-92 × 13-21	55-82 × 20-27
Shape	Fusoid, obclavate fusoid, straight or curved	Fusoid, obclavate fusoid, straight or curved	Fusoid, obclavate fusoid, straight or curved	Fusoid, obclavate fusoid, straight or curved	Tapering at both ends, thick-walled
Color	Light hyaline	Hyaline	Hyaline	Hyaline	Brown to dark brown
Germination %*	99.8	99.5	98.8	97.5	1.0
Mode of germination	Bipolar	Bipolar	Bipolar	Bipolar	Unipolar
Septation	Euseptate (8- 10)	Euseptate (8-10)	Euseptate (8-10)	Euseptate (8-10)	Distoseptate (3-5)
Pathogenicity	+	+	+	+	+
Incubation period	5 days	5 days	5 days	5 days	5 days
No. of lesion/cm^2	11.5	7.0 (large)	2.5	2.5	2.5

*Mean of 250 conidia at 25°C.

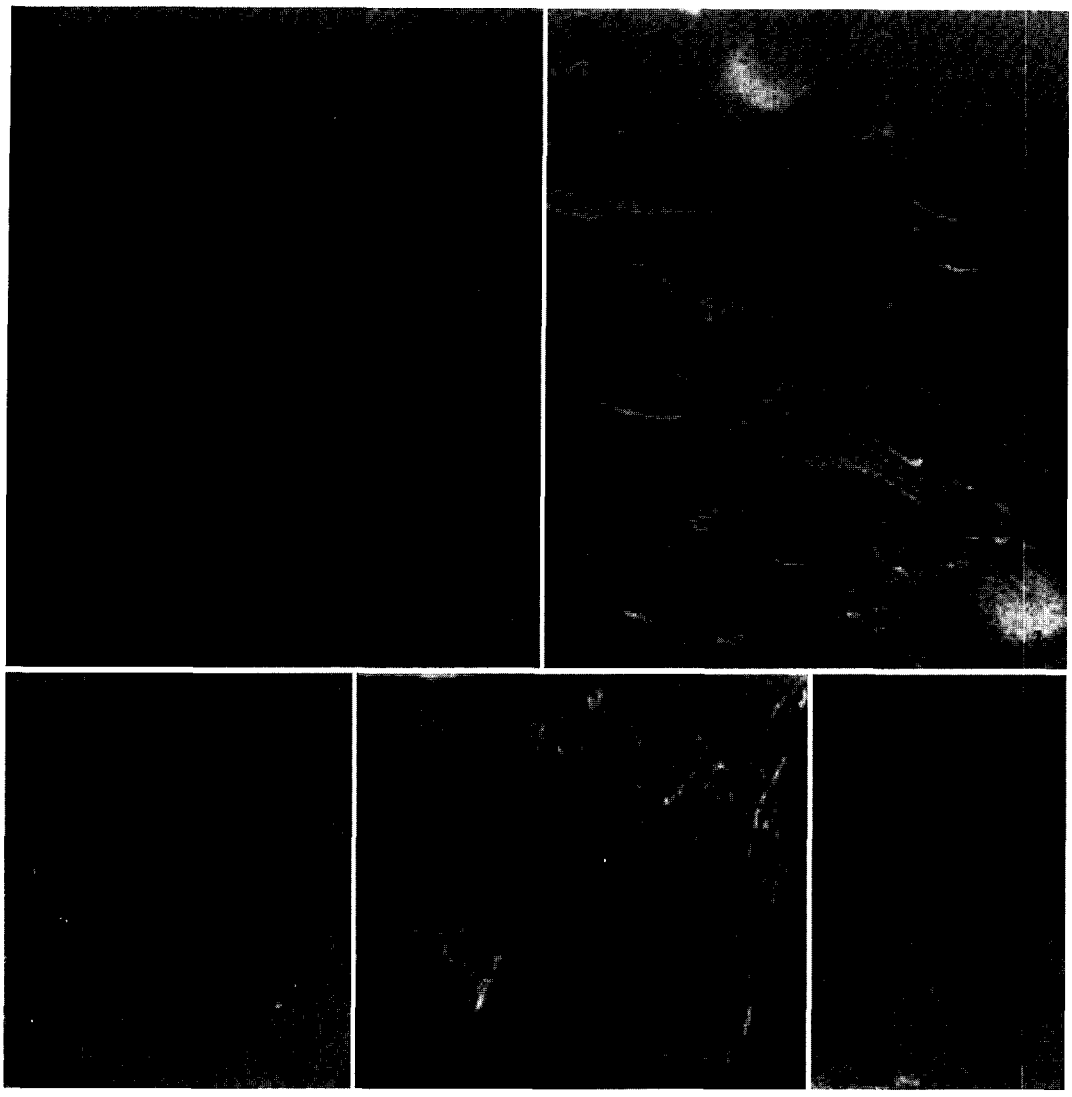

Fig. 1. Type of conidia observed on living diseased plant hosts in the field (a); Conidia type on PDA medium (b); Conidia aggregate also referred to as clump of conidia in this study (c); Conidia detached from clump of conidia (d); Resting type of conidia from spore clump (e).

Table 2. Character changes of *Bipolaris sorokiniana* conidia during wheat straw storage in sealed paper packets at room temperature

Character	Storage duration (month) and morphological/ physiological changes				
	1	2	3	4	5
Size	No change	No change	Changes started	Parasitic conidia completely replaced by the spore clumps	No change
Shape	Fusoid, obclavate fusoid, rarely truly cylindrical straight or curved	Fusoid, obclavate fusoid, rarely truly cylindrical straight or curved	Fusoid, obclavate fusoid, rarely truly cylindrical straight or curved	Boat shaped conidia produced in spore clump	Boat shaped conidia produced in spore clump
Color	Hyaline	Hyaline	Hyaline to dark	Dark conidia	Dark conidia
Occurrence of resting type of conidia	Absent	Absent	Production initiated	Production ending	Only saprophytic conidia present
Clump formation	Absent	Absent	Initiation	Aggregates formation completed	No change in aspect

clearly consisted of large numbers of shorter brown to dark-brown conidia similar to those produced on PDA (Table 2, Fig. 1c). After their formation, these conidia aggregates easily detached themselves from the plant tissue and fell at the bottom of the envelope.

At harvesting time, an overwhelming majority of conidia observed on infected seeds were hyaline but a few dark conidia were also found. After storage, conidia isolated from external seed coats appeared dark, small, distoseptate, similar to the conidia produced on PDA, and showed a germination percentage close to 1% in the slide glass test.

Discussion

The survival of *Bipolaris sorokiniana* on seed is probably a major source of primary inoculum (Mehta, 1993). The survival of *B. sorokiniana* conidia in the soil is well documented and free dormant conidia can survive up to 37 months under aerobic conditions (Simmonds et. al., 1950; Lidingham and Chinn, 1955; Chinn et. al., 1960; Reis and Santos, 1987; Reis, 1991). In contrast, very little information is available on conidia survival associated with infected stubbles and straw. The present study showed that the pathogen might present two types of conidia corresponding to different physiological conditions. Conidia directly produced on living tissues are characterized by a rapid bipolar germination allowing the pathogen to complete several cycles of fast conidial multiplication during the cropping season.

The saprophytic survival of the pathogen occurs on plant debris after crop harvest. If the humidity increases, conidia present on crop residues germinate and may produce secondary saprophytic conidia similar to the conidia produced on PDA and characterized by a poor or slower germination. However, it is also possible that the conidia size shrinks under lower moisture condition, become darker in color, and enter in a dormant phase with reduced germination. These saprophytic conidia eventually aggregate under variable relative humidity or electrostatic conditions occurring inside the sealed packet leading to the fall of the fungal structure from the host. In this study, although the conidia produced on wheat straw and on PDA showed poor germination using the slide glass technique, their pathogenicity and incubation period were not reduced compared with those conidia produced during the cropping season on living host plants. The germination of saprophytically produced conidia was higher using inoculation on wheat plants unlike in the slide glass test. The reduced number of lesion density on wheat plants after inoculating with saprophytic conidia is likely due to the lower number of infection sites resulting from unipolar germination.

Saprophytic conidia can easily be distinguished from parasitic conidia by the dark color distoseptation and the thick cell wall (Luttrell, 1963). These features are possibly favoring a dormancy that enhances the conidia longevity during *B. sorokiniana* survival in the soil, plant debris, and seeds. Also, after inoculating soil with *B. sorokiniana* conidia produced on PDA, Boosalis (1962) noticed a very poor germination but the germination rate could be increased several folds using several substrate amendments.

Since the disease development is dependent on the initial amount of inoculum, the relative importance of infected seed, free conidia in the soil, and stubbles may highly vary according to cropping systems resulting in different effects on disease incidence and severity. Since the conidia germination can be induced by adding certain substrates (Boosalis, 1962), possibilities of biocontrol through suppressive environmental conditions may emerge. However, when fungicides are used as a seed treatment, they may prove ineffective due to the impermeability of dormant conidia or because the fungicide may prove ineffective until the germination is induced (Reis et al., 1998).

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