

Sources of *Cochliobolus sativus* Inoculum Causing Spot Blotch under Warm Wheat Growing Conditions in South Asia

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Wheat (*Triticum aestivum* L.) production in the warm areas of South Asia is severely affected by spot blotch caused by *Cochliobolus sativus*. There are several inoculum sources suspected to harbor *C. sativus* during the off season. This study was conducted to determine survival and potential sources of *C. sativus* inoculum from rice stubble, wheat seed, soil and weeds in three cropping systems (wheat-rice, wheat-fallow and wheat-green manure) during the 2004 and 2005 wheat growing seasons at Rampur, Nepal. Wheat seed, soil and rice stubble in the field, and weed plants in and around research plots were examined for the presence of the pathogen. Koch's postulates were applied to verify representative strains. The pathogen was found in the wheat seed at moderate to high levels, but was absent in soil samples and rice stubble collected from the field before wheat was sown after rice. Weeds such as *Blumea* sp., *Dichanthium annulatum*, *Digitaria ciliaris*, *Phalaris minor*, *Saccharum officinarum*, *Axonopus compressus*, *Brachiaria mutica*, *Eleusine coracana*, *Panicum* sp., *Paspalum scrobiculatum*, *Pennisetum purpureum*, *Setaria italica*, *Zea mays* and *Zizania aquatica* harbored *C. sativus*. The *C. sativus* strains isolated from these weeds infected wheat by producing typical spot blotch symptoms. It is concluded that the weeds prevalent in different cropping sequences and on adjacent uncultivated land can serve as secondary hosts for *C. sativus*. This study presents important new information that could assist in better understanding spot blotch epidemiology and in developing integrated management strategies for spot blotch of wheat in the warmer growing regions of South Asia.

Keywords: alternate host, *Bipolaris sorokiniana*, *Cochliobolus sativus*, source of inoculum, *Triticum aestivum*

Introduction

Several abiotic and biotic constraints affect wheat production in South Asia (Dubin and Duveiller 2000; Duveiller 2004). Among biotic constraints, the most important wheat disease in many parts of South Asia including the Nepal lowlands is spot blotch, caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur (anamorph: *Bipolaris sorokiniana* (Sacc.) Shoemaker) (Maraite et al. 1998; Saari 1998; Sharma et al. 2003b).

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Grain yield losses due to spot blotch have been reported to reach up to 20 to 30% in farmers' fields and at experiment stations in the region (Saari 1998; Sharma and Duveiller 2004).

A number of previous studies reported seed transmission of *C. sativus* in wheat (Neergaard 1977; Reis 1983; 1991; Sati et al. 1993; Goulart 1996). A recent study in South Asia has shown that seed is the primary, but not the only, source of inoculum triggering a new disease cycle early in the wheat crop season (Duveiller et al. 2005). Only limited studies have been conducted in the warm wheat growing climate to elucidate other sources of spot blotch inoculum (Pandey et al. 2005). Seed treatments with chemicals have shown excellent control of seed-borne inoculum of *C. sativus* in the rice-wheat system in South Asia (Sharma-Poudyal et al. 2005). However, even with the use of seed treatments, these studies reported early wheat crop infection with spot blotch suggesting the existence other sources of inoculum.

Limited studies in the Americas conducted in cropping systems other than the rice-wheat system used in South Asia have shown that crop debris (Reis 1991) and soil (Duczek 1981; Mathieson et al. 1990; Tinline and Spurr 1991) harbor the pathogen in fields where the previous crop was wheat that had been infected by *C. sativus*. Crop history could play an important role in the distribution of conidia in the soil (Piening and Orr 1988; Wildermuth and McNamara 1991). Bakonyi et al. (1998) listed a number of weeds and other crops from which *C. sativus* was isolated in Hungary. Pandey et al. (2005) isolated *C. sativus* from three of 22 weed species present in a wheat field under a rice-wheat cropping sequence in eastern India. Saari (1998) pointed out that the most widely used rice-wheat cropping system in South Asia provided a favorable environment for the survival and multiplication of *C. sativus*. Misra (1973) also suggested that rice stubble may be a substrate for the fungus.

Studies conducted in other parts of the world showed that seed transmission is an important source of primary inoculum for *C. sativus* (Goulart 1996). However, such information is not available for the hot wheat growing conditions in South Asia where spot blotch is considered more important than other wheat foliar diseases. The present study was conducted to determine the respective roles of seed, rice stubble, soil and weed flora, as sources of *C. sativus* inoculum in the warm climate rice-wheat cropping system of South Asia.

Materials and Methods

Three adjoining fields with different annual cropping systems were studied: wheat grown after rice, wheat grown after green manure using *Sesbania aculeata*, and wheat grown after wheat with a fallow period in summer. The experiment was conducted in the 2003–2004 (2004) and 2004–2005 (2005) wheat seasons on the experimental farm of the Institute of Agriculture and Animal Science, Rampur, Nepal (27°40' N and 84°19' E; 228 m above sea level). In each cropping system, the experiment was laid out in a randomized complete block design with three replicates. The soil type at the test site was a medium-textured loam and spot blotch epidemics on wheat had been severe for the previous

several years. The research site is representative of the warm humid climate that occurs in the eastern Gangetic plains.

Isolation of C. sativus from rice stubble

After the rice harvest and prior to land preparation for wheat seeding, 100 samples of rice stubble, approximately 10 to 20 cm long, were collected in a diagonal transect of a field managed as a wheat-rice cropping system. The bulked samples were washed with tap water, cut into 160 × 2 cm long pieces, surface sterilized with 0.5% sodium hypochlorite (NaOCl) for 2 minutes, rinsed twice with distilled water and placed on water agar in Petri dishes following the procedure described by Tinline (1977) and Whittle (1977). Streptomycin was used to control bacterial growth. Ten stubble pieces were placed in each Petri dish and incubated at $25 \pm 1^\circ\text{C}$ under a cycle of 12 h light and 12 h darkness (Sharma et al. 2003b). Observations were taken after 48 h and one week using a stereomicroscope when fungi were sub-cultured for purification on PDA. The putative *C. sativus* cultures were maintained in PDA tubes for subsequent confirmation.

Isolation of pathogens from soil

For each field in the three cropping systems, soil samples were taken at 10 different points along a diagonal transect during land preparation prior to wheat seeding and at tillering and flowering of the subsequent wheat crop. The soil samples were collected with the help of a bucket auger 7.1 cm in diameter and to a depth of 20 cm. The 10 soil samples collected at each growth stage in each cropping system were pooled. Thus there were nine composite samples for three growth stages and three cropping systems. One gram of soil from each of the nine samples was diluted in water to 10 ml volume or a 10^{-1} dilution from which 10^{-2} and 10^{-3} dilutions were made. An aliquot of 0.1 µl from the 10^{-3} dilution was plated in Petri dishes containing a starch-nitrate agar semi-selective medium (Dodman and Reinke 1982) with five replications. The Petri dishes were incubated at 25°C under a cycle of 12 h light and 12 h darkness. Plates were observed daily to check the growth of the pathogen, and mycelia from individual colonies were transferred to PDA plates for purification and identification of *C. sativus*.

Isolation of pathogens from seed

Three wheat varieties (Sonalika, BL1473 and Milan/Shanghai#7) earlier identified to have different levels of spot blotch response (Duveiller et al. 2005) were used in the study. Twenty-five seeds were placed separately in sterile Petri dishes containing a double layer of sterile moist blotting paper. The plates were incubated under 12 h light : 12 h darkness at 20°C for 5 to 7 days. A set of four Petri dishes per variety was considered as one experimental unit and replicated four times. The seeds were observed under a binocular stereomicroscope, and numbers of seeds infected with *C. sativus* were recorded together with the number of germinated seeds.

Isolation from putative weed hosts

Weed leaves showing symptoms similar to spot blotch were collected from fields undergoing different cropping sequences (wheat-rice, wheat-fallow, and wheat-green manure) as well as from uncultivated land surrounding the fields prior to wheat seeding, and in the standing wheat crop at the maximum tillering and flowering stages. The weed leaves were cut into 2 cm pieces and surface sterilized with 0.5% NaOCl for 2 minutes. The samples were rinsed twice with sterile distilled water and put in Petri dishes containing 2% water agar following published procedures (Tinline 1977) with 10 pieces in each plate. Three plates per weed species were incubated at 25°C under a 12 : 12 h light : darkness regime (Sharma et al. 2003b). The samples were observed after 48 h using a stereomicroscope and individual conidia suspected to be *C. sativus* were transferred to PDA tubes for further identification. The weed samples were also preserved as herbarium specimens.

Pathogenicity tests

For pathogenicity tests, the isolates taken from different weed hosts were inoculated to seedlings of spot blotch susceptible wheat variety Sonalika raised in metallic trays in the greenhouse. The trays (37.5 cm × 26.5 cm and 11.5 cm height) were previously surface sterilized over a flame. Soil was sterilized by autoclaving at 121°C for 2 hours. Each tray was filled with 5 kg sterilized soil. Ten seeds, treated with 0.5% mercuric chloride for 10 minutes, were sown in a tray; four trays were used as four replicates. The isolates obtained from different weed species were sampled from stock cultures and grown in PDA test tubes for 10 days at room temperature (22–24°C). These test tubes were kept for 5 days under near UV light that allowed formation of profuse conidia. Conidial suspensions were prepared by taking conidia from the PDA agar surface and mixing them in 250 ml sterile distilled water in a flask. The conidial suspension was filtered through cheesecloth to remove mycelial and agar fragments, and adjusted to 10^4 conidia/ml (Gilchrist 1985) with one drop of Tween-20. Inoculations were made by spraying 21-day-old wheat seedlings with a spore suspension using a hand sprayer (Hossain and Azad 1992). The inoculated trays were put in a humid chamber for 36 h and then transferred to a mist chamber for disease development.

Symptoms were observed on the plants 12 days after inoculation. Lesion lengths on wheat leaves were measured for different isolates to determine if the weed source had any influence on the virulence of the pathogen on wheat. Leaves showing symptoms were cut and surface sterilized with 0.5% NaOCl, rinsed with sterile distilled water and incubated at 25°C in sterile Petri dishes containing a double layer of sterile moist blotting paper under 12 : 12 h light : darkness conditions (Sharma et al. 2003b). Conidia production after 48 h was observed under the stereomicroscope.

Results

Cochliobolus sativus was absent in rice stubble in both years. Also, *C. sativus* was not detected in soil samples collected prior to wheat seeding under all three cropping sequences

in both years. However, *C. sativus* was detected in the soil at the later growth stages of the wheat crop (Table 1). Among the three wheat genotypes, seed infection with *C. sativus* was high in BL1473 (96%) and Sonalika (94%) and moderate in Milan/Shanghai-7 (45%) in 2004 (Fig. 1). In 2005, seed infection levels were 94, 93 and 83%, respectively. Milan/Shanghai-7 had the highest germination followed by BL1473 and Sonalika.

All isolates of *C. sativus* collected from weed hosts were pathogenic to wheat (Table 2). The pathogen was isolated from *Blumea* sp., *Dichanthium annulatum*, *Digitaria ciliaris*, *Phalaris minor* and *Saccharum officinarum* growing within the crop fields and *Axonopus compressus*, *Brachiaria mutica*, *Eleusine coracana*, *Panicum* sp., *Paspalum scrobiculatum*, *Pennisetum purpureum*, *Setaria italica*, *Zea mays* and *Zizania aquatica* collected in surrounding areas. There were large differences in the sizes of the lesions produced by isolates from different weed hosts.

Table 1. Presence of *Cochliobolus sativus* in soil before and after sowing of wheat in a wheat based cropping system in 2004 and 2005 at Rampur, Nepal

Cropping system	Before sowing		Maximum tillering		Flowering stage	
	2004	2005	2004	2005	2004	2005
Wheat – rice	–†	–	+	+	+	+
Wheat – fallow	–	–	–	+	+	+
Wheat – green manure	–	–	–	+	+	+

† – and + represent absence and presence of *C. sativus*, respectively

Table 2. Weed hosts harboring *Cochliobolus sativus* in different cropping sequences and on uncultivated land in the vicinity of wheat crops in 2004 and 2005 at Rampur, Nepal

Weed host	Source of sample	Lesion length (mm)‡	
		2004	2005
<i>Axonopus compressus</i>	Vicinity of research plots	7*	na†
<i>Blumea</i> sp.	Wheat – rice	7	na
<i>Brachiaria mutica</i>	Vicinity of research plots	na	1
<i>Dichanthium annulatum</i>	Wheat – green manure	5	na
<i>Digitaria ciliaris</i>	Wheat – green manure	3	na
<i>Eleusine coracana</i>	Vicinity of research plots	4	11
<i>Panicum repens</i>	Vicinity of research plots	6	7
<i>Panicum</i> sp.	Vicinity of research plots	7	7
<i>Paspalum scrobiculatum</i>	Vicinity of research plots	5	7
<i>Pennisetum purpureum</i>	Vicinity of research plots	1	na
<i>Phalaris minor</i>	Wheat field	1	10
<i>Saccharum officinarum</i>	Wheat – green manure	6	na
<i>Setaria italica</i>	Vicinity of research plots	11	11
<i>Zea mays</i>	Vicinity of research plots	na	2
<i>Zizania aquatica</i>	Wheat field	na	2

‡ *C. sativus* isolated and confirmed

* Average length of the lesions produced on susceptible wheat genotype by *Cochliobolus sativus* isolates from different weed hosts

† na sample not available

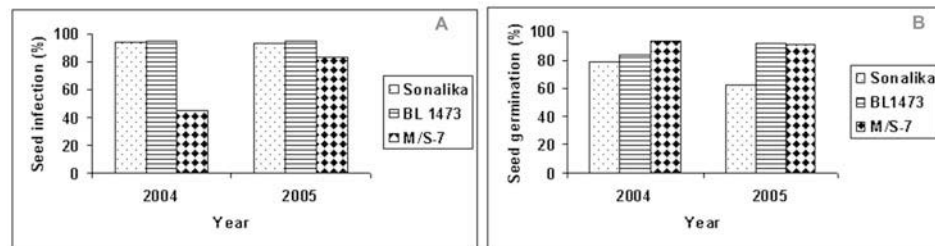


Figure 1. Seed infection (A) by *Cochliobolus sativus* and seed germination (B) of three wheat genotypes prior to seeding in two years at Rampur, Nepal. The bars show the standard error for individual mean values (n = 16)

Discussion

The results of this study suggest that *C. sativus* did not survive in rice stubble. This finding agrees with the previous observation of Alam et al. (1994). Our results do not support the hypothesis that rice might serve as a host species for the spot blotch pathogen, and that rice stubble could harbor inoculum attacking wheat in a rice-wheat cropping system (Misra 1973). This hypothesis was based on research where there was cross inoculation of rice with both *C. sativus* and *P. tritici-repentis* from wheat (Misra 1973). Moreover, if rice acted as a green bridge it should be possible and easier to detect the wheat pathogens on rice leaves in the rice crop preceding wheat rather than finding them on rice stubble at the time of sowing wheat. We have not observed spot blotch on rice preceding wheat.

Failure to detect the pathogen in soil samples prior to wheat seeding but its presence in the soil at the later growth stages of the wheat crop suggests that *C. sativus* may be in the soil, but it does not survive the several weeks of monsoon flooding that is typical of paddy fields in the region. This finding differs from a previous report where conidial survival in the soil served as a primary source of inoculum for the following wheat crop (Reis and dos Santos 1987). The prolonged flooded conditions at relatively high temperatures could account for such difference.

High levels of infection of the seeds by the pathogen suggest that infected seeds do serve as a primary source of *C. sativus* inoculum, confirming previous findings of Sharma et al. (2003a, 2005) and Sharma-Poudyal et al. (2005). Differences between varieties in the levels of seed infection in 2004 were attributed to different sources of seed. The level of seed infection is influenced by the extent of spot blotch severity in the wheat field where the seed is produced (Sharma et al. 2005). Varietal differences in seed infection level were lower in 2005 because seed of the three varieties came from the same 2004 field.

Isolation of the pathogen on the weed hosts suggests that weeds around the wheat fields serve as alternate hosts for *C. sativus* during the off season. This finding only partly agrees with those of Bakonyi et al. (1998), but that study was conducted under very different conditions in Europe. Two previous studies in South Asia reported weed hosts of *C. sativus* in the rice-wheat cropping system (Singh et al. 1998; Pandey et al. 2005). Singh et al. (1998) did not conduct pathogenicity tests on wheat. Pandey et al. (2005) isolated the pathogen only from *Setaria glauca*, *Echinochloa colonum* and *Pennisetum typhoides* in a

rice-wheat systems area in eastern India, but the isolates did not infect wheat, leading to a conclusion that infected weed hosts were not primary sources of inoculum. Thus we present new evidence that weeds do serve as secondary hosts for *C. sativus* and as primary inoculum sources for wheat in the warmer wheat-rice cropping systems of South Asia.

Previous studies conducted in other parts of the world showed that foliar blight fungi infecting wheat have secondary weed hosts and can survive in soil, crop debris, volunteer plants, and as seed borne infections (Raemaekers 1988; Reis 1991; Duveiller and Gilchrist 1994; Schilder and Bergstrom 1995). However, comprehensive information on the primary sources of inoculum in the warmer wheat growing conditions of South Asia is lacking. The present study conducted over two years attempted to identify possible primary inoculum sources in the wheat-rice system in the eastern Gangetic plains of Nepal. Infected seed is the most important primary source of inoculum. Neither crop debris nor soil serves as a source of primary inoculum for *C. sativus* in this region. A number of grass weeds occurring in and around cropped fields served as secondary hosts to *C. sativus* and we presume these could be a source of inoculum to wheat. Rice did not act as a host and rice stubble did not support the survival of inoculum, possibly due to the annual monsoonal flooding of fields and high temperatures that ensure the break-down of debris. This study provides new information towards understanding the epidemiology of spot blotch in South Asia. The findings underline the importance of weed control in developing an integrated spot blotch management strategy for wheat in the warm growing regions.

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