Journal of Agrometeorology 8(2) : 215 - 222 (Dec. 2006)

Dispersal of secondary inoculum of *Tilletia Indica* causing karnal bunt of wheat in relation to weather parameters

GURBIR KAUR, SATVINDER KAUR and S. S. HUNDAL
Punjab Agricultuiral University, Ludhiana

ABSTRACT

The present investigation on dispersal of the infective stage of *Tilletia indica*, was observed up to 50cm aerially (with wind), up to 25 cm with rain drops of 5000um (using single droplet simulator) and during drizzle (rain drop size of 200um) there was no splash of sporidia upwards when an actively growing colony of *Tilletia indica* was incubated in the wheat field. Significant differences (-0.73) were observed in the number of sporidia formed on the soil surface and those moving upwards at different heights and on different days in the wheat field indicating a strong influence of weather. Maximum number of sporidia were trapped at 50 cm above the ground and maximum number (15/200x) was obtained on 14th Feb, when temperature for the day varied between 8.6-22 °C and relative humidity 47-97 percent. Overall maximum sporidia (35/200x) were observed at 30 cm height. There was significant reduction in the number of sporidia trapped in March. A significantly negative correlation - r (at 5 an 1 %), was obtained with daily prevalent relative humidity, air and soil temperatures and to some extent with sunshine hours and poorly correlated with minimum and maximum temperature and these factors are also known to affect the number of sporidia produced. A third degree polynomial (R^2 =0.93) explained the variation in the number of sporidia trapped at different heights

Key Words : Dispersal, rain, secondary sporidia, *Tilletia indica*, weather parameters

To understand the disease epidemiology, in addition to host parasite interactions, each and every stage of the pathogen life cycle also needs to be examined with respect to its interaction with the weather conditions (Aujla *et al* 1996). Secondary (or allentoid) sporidia constitute the infective stage of *T. indica,* which infect the developing ovaries of wheat flower. According to Bedi (1949) and Munjal (1971), secondary sporidia were produced on small microscopic colonies formed by the germinating teliospores on soil in the wheat field, which are discharged forcibly and get air-borne and infect the developing ovaries. Their dispersal and deposition of the viable sporidia on the wheat ears and coincidence with the susceptible stage of the host was the most important factor in the occurrence of Karnal bunt of wheat in the endemic areas where soil-borne telial inoculum was available. However this stage is critically affected by weather (Kaur *et al* , 2002, 2005). The present study was designed to define the events and conditions in the wheat field which initiate and affect the dispersal of secondary sporidia of *T. indica* , which were trapped in the wheat crop at different heights and extent of dispersal was correlated with weather parameters.

MATERIAL AND METHODS

Aerial dispersal

Experiments were carried out at Punjab Agricultural University Ludhiana in the experimental area during wheat crop season 2003-05. Agronomic conditions were maintained as per the recommendations for the cultivation of irrigated normal sown wheat. Number of sporidia formed by the colony (0 cm) and that reached 10, 20, 30, 40, 50, 60, 90 cm above ground were estimated with spore traps, made from glass slides of 7.5 cm X 2.5 cm, thoroughly coated with vaseline, fitted on wooden stands and placed around the source of sporidial inoculum within the field. These spore traps were examined after 24 hrs. of exposure at 200x. Experiment was carried out incubating primary inoculum (teliospores) in the field on soil plates in 3 replications, from November 2002 till March 2003 and repeated at 15 day intervals. But tracking germinated teliospores was found to be impractical and no sporodia were observed on the spore traps. This is because under natural conditions teliospore germination was effected by complex factors (Kaur and Kaur 2005). The experiment was redesigned the following year by incubating the actively growing colonies of *T.indica* (weighing 50 grams), which were grown in the laboratory and the experiment was repeated 7 times (Feb.-March 2004) during the susceptible stage of the wheat crop, growth stage 46- 65 (awn emergence to post anthesis). The colony was washed with sterile water, kept floating on water in 8 x 8 inch plastic trays of 1 inch height and 5 replications were incubated each time in the field. The number of sporidia trapped were daily counted (after 24 hrs) and mean for 3 days (viz., 24, 48 and 72hrs.) was used for correlating with cumulative weather parameters. The experiment was repeated at 7 days interval. The data were analysed using factorial RBD to determine the critical difference and coefficient of correlation between the sporidia trapped on different dates during heading of wheat and correlation (r) with daily weathr parameters was calculated and best fit polynomial equation developed and R^2 value was calculated in a multiple step wise analysis. The wind conditions were calm and only sub canopy passive air currents were prevalent during the period of investigation.

Splash dispersal

An experiment was designed to study the height to which the sporidial inoculum will reach on splash dispersal. Three week old *T. indica* cultures, grown on YPDA were used and inoculum was prepared in sterilized water by adjusting the spore concentration to 50,000 sporidia per ml. A trough with inoculum was placed at 2 cm and 20 cm height above ground in the

Table 1 : Number of sporidia trapped at different heights from colonies of *Tilletia indica* placed in the wheat crop during Feb-Mar, 2004, mean weather conditions

Date	Spore Trap cms/ no. sporidia at height of						Weather parameters (mean of 72 hrs)							
	0	10	20	30	40	50	RH -M	RH -E	T_{Max}	T_{Min}	ST-E	ST-M	ST_{mean}	SSH /day
1st Feb.	108	25*	22	26	19	12	98	60	17	5	19	6	12	9
7th Feb.	103	20	25	35	21	14	87	49	21	9	23	9	16	7
14th Feb.	102	18	29	30	25	10	98	45	23	8	23	10	17	10
21st Feb.	62	8	9	7	9	4	96	46	25	10	28	11	19	11
28th Feb.	65	15	8	9	0	2	98	41	27	11	30	14	22	11
7th Mar	27	4	5	3	0	0	81	45	29	14	33	16	24	11
14th Mar	23	5	1	0	0	0	95	48	32	16	40	18	28	10
Mean	70	13.6	14.1	15.7	10.6	6	93.3	47.7	24.9	0.43	28.0	12	19.7	9.9

CD 5% : Height = 1.06, Dates = 1.15, Height x dates = 2.81

Coefficient of correlation : Height and mean sporidia trapped $r = -0.73$

*Average number of sporidia/ 200x on trap slides, T= Air Temperature maximum and minimum

ST= Soil temperature E= evening M =morning SSH =Sun shine hours

wheat field and water drops simulating drizzle (200 mm in diam.) and heavy rain (5000 mm in diam.) were dropped using "Single Raindrop Simulator" (Chaudhary *et al*, 1978). The height to which the inoculum reached on splash dispersal was recorded.

RESULTS AND DISCUSSION

Wind dispersal

Significantly more number of sporidia were formed by the colony on the soil surface than detected at different heights ($r = -0.73$) and the differences varied with (a) date of incubation of the colony (b) number of sporidia produced on the soil and (c) height of spore trap above the ground and confirms earlier findings (Sidhardha *et al* 1996).

The influence of the prevalent weather conditions on the number of viable sporidia. detected in temporal and vertical dimensions, was important. Maximum number of sporidia were dispersed up to 30 cm, when temperature varied from 8 to 23°C . Vertical distribution from 0-50 cms correlated negatively with daily prevalent relative humidity, air and soil temperatures and to some extent with sunshine hours and poorly correlated with minimum and maximum temperature measured (Table 2). It has been reported that at the soil surface, minimum and maximum temperature had significant effect on the number of sporidia produced and correlation was much less with morning and evening RH (66-100%) in irrigated wheat fields of Punjab (Kaur *et al* 2002 , Kaur and Kaur 2005). In the present investigations higher number of sporidia (22-23 per 200x field of microscope) were trapped from 7-14th Feb, 2004 when the mean air temperature varied from 8 °C (T_{min}) to 23 °C (T_{max}) and RH from 45

Table 2: Sporidia trapped at different heights and correlation with daily weather parameters:

Height cms	Weather parameters with number of sporidia dispersed							
	T_{max}	T_{min}	$RH_{morning}$	$RH_{evening}$	$ST_{evening}$	$ST_{morning}$	ST_{mean}	SSH
0	-0.95	-0.83	0.72	0.61	-0.95	-0.88	-0.34	-0.95
10	-0.84	-0.71	0.18	0.37	-0.74	-0.84	-0.83	-0.43
20	-0.79	-0.73	0.13	0.21	-0.79	-0.84	-0.85	-0.45
30	-0.79	-0.70	0.07	0.22	-0.78	-0.83	-0.84	-0.35
40	-0.78	-0.72	0.15	0.29	-0.78	-0.83	-0.84	-0.42
50	-0.88	-0.72	0.07	0.40	-0.81	-0.88	-0.88	-0.37
Mean	-0.84	-0.74	0.12	0.29	-0.81	-0.87	-0.88	-0.53

r (at 5%) = 0.43 r (at 1%) = 0.55

Table 3 : Splash dispersal of sporidial inoculum of *Tilletia indica* with heavy rain and drizzle

Droplet size?/ number of droplets*/ht ?	5cm	15cm	20cm	25cm
5000µm (Heavy rain)**	>8*	6-8*	4-5*	1-2*
200µm (Drizzle)	0	0	0	0

**Droplets released from 3m height using single raindrop simulator

to 98%. This differed significantly from 1st Feb when temperature varied from 5-17 °C and RH 60-98% when number of sporidia were 20 per 200x. With the overall rise in temperature, the number of sporidia produced on the soil was reduced and a significant reduction in the vertical movement of the sporidia trapped was observed. Regression functions indicated a significant relationship with third degree polynomial in stepwise multiple regression analysis, which explained about 96% of the variation observed, no.of sporidia (Y) : $Y = 25.04 + 0.036\, RH_{max} + 0.032\, RH_{min} + 0.37\, T_{max} + 0.50\, T_{min} + 0.95 S T_{min} + 2.18 S T_{max} - 1.34\, ST_{mean} - 4.89\, SSH$ and the R^2 was 0.93

The sporidia reaching the wheat ears during the susceptible window of the host was converted into number /ml as : (X / 0.01677) x 1000, (where X is number of sporidia / microscopic field 200x on the trap slide), to compare with the threshold required (50,000 sporidia/ml under experimental conditions ,Gill *et al* 1992, Kaur 2004 = 84-85 sporidia / 200x.) for the severe disease development. It was observed to vary from 0 to 8944 sporidia / ml and was much less as compared to the requirment. Hence the weather conditions of 2003-04 on the basis of quantitative data on the dispersal of viable sporidia from an actively growing colonies kept on the soil surface to the ear head were not favorable for severe disease development. The disease in the farmers field was also found to be less in the subsequent market surveys (average in Punjab = 0.024%, 2005) validating our observation on the dispersal of sporidia observed. More sporidia are formed and released at night (Kaur *et al.* 2002), when in winters wind conditions are calm the sporidia may more upwards. But this may be affected by (a) obstacles due to the foliage and crop density and (b) unfavourable weather conditions which affect the number formed and upward movement of viable sporidia . These constraints affect the disease incidence on the farmer's field inspite of the telial inoculum of T.*indica* having germinated and formed abundant sporidial ccolonies on the soil. That may be one of the reasons why eavily tillering genotypes like PBW 343, which have been found to be less affected by the disease in the field and contributed to the declining trend of the disease over the years in Punjab (Kaur *et al* 2005). However under continuously favourable weather conditions the pathogen will overcome this impediment by producing more sporidia and jumping up stepwise in the crop canopy. In addition the sporidia from the *T.indica* colonies present on the edge of the field will be more uplifted in view of the forcible discharge (being blastospores), as these will get shot above the laminar currents and enter the turbulent wind (Rosenberg 1974), reach the wheat spikes, get trapped in or may at least get deposited on the upper leaves. Uneven distribution of the infected wheat spikes in the farmers field can be thus explained (Singh 1993).

Splash dispersal

Heavy rain (having droplet size of 5000 mm) dispersed sporidia to maximum of 25 cm above soil surface, whereas maximum number of droplets were recorded at 5 cm height (Table 3). These studies are in agreement with Fitt *et al* (1989) who reported that only heavy raindrops contribute towards the splash dispersal of inoculum. Spells of short duration heavy rain prior to heading may help in a series of small jumps, from the soil surface, where the germinated colonies of *T. indica* are present enabling monkey jumping of the infective stage (Nagarajan *et al* 1997). But continued heavy rainfall may also wash off the secondary inoculum which has climbed up to some level. That is why less disease was observed in the years when heavy rainfall coincided with heading (Singh 1994).

On the basis of present investigations the role of rain appeared to be multifarious such as helping to disperse, survive or even having a washing down effect from the plant surface on the sporidial inoculum of *T. indica* depending upon the timing and intensity of rainfall. The drizzle (having droplet size of 200 mm) may not directly play a significant role in the splash dispersal of the sporidial inoculum from the soil surface to the wheat ears, but :

(i) Spells of drizzle during heading of the wheat crop do help in maintaining the optimum temperature and saturated air atmosphere. This is important because sporidial inoculum tends to dehydrate as individual sporidia were observed to coil and shrink to very small size during the day when the surfaces (leaf and ear head) were dry and part of the sporidial inoculum becomes ineffective. Frequent drizzle during heading helps to maintain saturated air atmosphere and prevents dehydration of secondary sporidia (Kaur *et al* 2002).

(ii) The spells of drizzle at boot leaf stage will help to wash and collect the inoculum as drops in the auricle of the boot at the point where the wheat ear was about to emerge and the water droplet containing the secondary sporidia can get sucked into the boot and cause infection (Nagarajan 1991), which was later demonstrated (Kaur and Kaur 2005). Hence along with rain water splashes, drizzle, the dew drops may also assume importance. In Punjab conditions, the dew drops may remain on leaf surfaces till 11 am even on clear sunny days till the day temperature rises in the irrigated crop. The dew must be helping in the survival of secondary sporidia on flag leaf at GS-47 as those collected above the auricle of the boot leaf may get sucked in due to the vaccum inside the boot. The rain showers early in the wheat crop season may help in up-lifting the soil borne secondary sporidia from germinated teliospores and colonies of *T. indica* and initiate the process of the upward movement by monkey jumping from lower to upper leaves by the successive generations of secondary inoculum. The first jump from the soil was up to 25 cm, which is likely to occur in free spaces within the crop stand while it was a little higher on the edges.

Overall the present investigations indicate that the infective inoculum of *Tilletia indica* employs diverse means to reach the susceptible host tissue and both aerial dispersal of sporidia to the emerging ears and droplet sucking mechanisms of the boot at awn emerging stage may be operating (Kaur 2004; Kaur and Kaur 2005). The prevalent weather conditions had a significant influence on the number of sporidia formed and that moved upwards and on a normal cold winter day during heading of wheat in the northern part of India, where the disease was endemic but remained sporadic. More disease observed in fields where wheat crop lodges on heading in otherwise unfavourable years, validates our observation that the direct aerial spread of the sporidia from the colonies on the soil surface to the wheat ears was constrained and in the lodged crop the ears become more accessible. Spray inoculations simulate the wind aided deposition of sporidia on the emerging ears (which remain susceptible from growth stage 51 to 65, i.e. awn emergence to post anthesis). The sporidia deposited on flag leaf can also flow in to the boot or get collected above the boot as drop let, on account of dew or drizzle and can be sucked (Kaur and Kaur 2005). This will remain viable and effective for a longer period as secondary sporidia are known to multiply within the boot and infect more number of the developing ovaries. But the gradual upward dispersal of the inoculum from the sporidial colonies by forming generations on the host surface was constrained due to the reducing size of the subsequent generations in addition to dehydration and coiling depending upon whether the surface was dry, moist or hydrated (Kaur *et al* 2005, Kaur 2002). However in present investigations monkey jumping of sporidial inoculum was not ruled out under favourable conditions for the pathogen.

REFERENCES

Aujla, S. S., Kaur, S. and Gill, K. S. 1996. Darkness – a predisposing factor for enhanced karnal bunt infection in wheat. *J. Res. PAU,* 32 : 51-53.

Chaudhary, T. N., Jalotra, S. K. and Sood, M. C. 1978. Development of a hypodermic-needle type Rainulator. *J Indian Soc. Soil. Sci.,* 26 : 87-89.

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

Fitt, B. D. L., McCartney, H. A. and Walklate, P. J. 1989. The role of rain in dispersal of pathogen inoculum. *Ann Rev. Phytopath.,* 27: 241-70.

Kaur, S., Hundal, S. S., Kaur, G., Singh, K. and Pannu, P. P. S. 2005. Probability of Karnal bunt occurrence in Punjab. *J. Res. PAU,* .42: 139-147

Kaur, G. and Kaur, S. 2005. Effect of soil and weather on the germination of *Tilletia indica* teliospores. *Indian J. Agric. Sci.,* (Accepted).

Kaur, G. and Kaur, S. 2005. Simulating natural infection of wheat ovaries with *Tilletia indica* and interaction with date of sowing and varieties. *Crop Improvement,* 32 : 26-34

Kaur, G. 2005. *Dispersal mechanisms of secondary inoculum of Neovossia indica (Mitra) Mundkur(Syn. Tilletia indica Mitra).* Ph.D. dissertation, Punjab Agriculture University, Ludhiana.

Kaur, S., Singh, M. and Singh, K. 2002. Factors affecting telial and sporidial inoculum of *Neovossia indica. Indian Phytopath.,* 55:14-18.

Munjal, R. L. 1975. Studies on spore germination and control of *Neovossia indica*, the causal agent of Karnal bunt of wheat. *Indo-USSR Symp Wheat Diseases,* Krasnodar.

Nagarajan, S. 1991. Epidemiology of Karnal bunt of wheat incited by *Neovossia indica* and an attempt to develop a disease prediction system.*Technical Report CIMMYT*, Mexico.

Nagarajan, S., Aujla, S. S., Nanda, G. S., Sharma, I., Goel, L. B., Kumar, J. and

Singh, D. V.1997. Karnal bunt (*Tilletia indica*) of wheat – a review.*Rev Pl Pathol* 76: 1207-14.

Rosenberg, N. J. 1974. Wind and turbulent transport In: *Microclimate: The Biological Environment*. A Wiley-Interscience publishers, USA.

Sidhartha, V. S., Singh, D. V., Srivatava, K. D. and Aggarwal, R. 1995. Some epidemiological aspects of Karnal bunt of wheat. *Indian Phytopath.*, 48: 419-26.

Singh, A. 1994. Epidemiology and management of Karnal bunt disease of wheat. *Res Bull No.* 127, Directorate of Expt Station, G.B.Pant University of Agriculture and Technology.

Singh, M. 1993. *Factors affecting the survival of Karnal bunt inoculum (Neovossia indica (Mitra) Mundkur)*. M. Sc. thesis, Punjab Agricultural University, Ludhiana, India.