FORECASTING POTENTIAL DISTRIBUTION OF SPOT BLOTCH IN WHEAT UNDER CLIMATE CHANGE SCENARIO IN INDO-GANGETIC PLAINS

जलवायु परिवर्तन के परिप्रेक्ष्य के अंतर्गत सिंधु गंगा मैदानी क्षेत्रों मे गेंहू में लगने वाले स्पॉट ब्लॉच रोग के संभावित वितरण का पूर्वानुमान

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FORECASTING POTENTIAL DISTRIBUTION OF SPOT BLOTCH IN WHEAT UNDER CLIMATE CHANGE SCENARIO IN INDO-GANGETIC PLAINS

By

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CERTIFICATE

This is to certify that the thesis entitled "**Forecasting potential distribution of spot blotch in wheat under climate change scenario in Indo-Gangetic plains**" submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Plant Pathology** is a record of *Bona fide* research work carried out by **Mr. Ali Viani (Roll Number 9769)** under my guidance and supervision. No part of thesis has been submitted for any other degree of diploma.

All the assistance and help or information received during the work on this thesis has been duly acknowledged.

Place: IARI, New Delhi

Date: 13/02/2014

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Dedication

This thesis is dedicated to my dear daughters, Bahareh and Farzaneh Viani, and my dear and beloved wife, Ayda Niktash, who have a key role in my life and for their moral support and constant help and sustained encouragement.

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CHAPTER-I INTRODUCTION

Wheat is a staple food of nearly 35 % of the world's inhabitants. Because of its increasing demand, the anticipated global wheat requirement at the end of second decade of 20th century varies from 840 (Rosegrant *et al.,* 1995) to 1,050 million tons (Kronstad, 1998). To accomplish the projected target, world's wheat production will have to enhance from 1.6 % to 2.6 %, annually (Rajaram, 1999). Accordingly, the average global grain yield would have to enhance from the existing 2.5 t ha^{-1} to 3.8 t ha^{-1}. Only 18 countries in the world meet target of producing average wheat grain yields of around 3.8 t ha^{-1} in 1995 whereby most of which are located in the Northern Europe (Anonymous, 1996). Of the worldwide 215 million hectares sown to hexaploid (*Triticum aestivum*) and tetraploid (*T. turgidum* var. *durum*) wheat, 95 million hectares (44 %) is in Asia and out of which, 62 million hectares attributed to wheat are located in China, India, and Pakistan (Singh *et al.,* 2004). Despite of such a vast area under the golden grains production, the food security as well as production stability are of paramount significance in most of the Asian countries as the majority of farmers are resource poor.

Wheat is the second most important food crop of the India, which contributes nearly one third of the total food grain production. About one tenth of the global production is contributed from India. Wheat cultivation has traditionally been dominated by the northern region of India. In India wheat is grown during winter season and major wheat producing states are Punjab, Haryana and Uttar Pradesh. India recorded all time high 94.88 million tons of wheat production from an area of 29.90 million hectare during 2011-12 (Wheat Directorate Annual Report, 2012-13). The country needs to produce 100 million tons of wheat by 2030 to feed the ever-growing population, which is a major challenge under changing climatic scenario. Therefore, concerted efforts are needed to intensify the research on enhancing the productivity in terms of per unit area on ecologically and economically sustained basis.

Wheat is a widely adapted crop. It is grown from temperate, irrigated to dry and high-rain-fall areas and from warm, humid to dry, cold environments. Undoubtedly, this wide adaptation has been possible due to the complex nature of the plant’s genome, which provides great plasticity to the crop. Wheat is a C3 plant and

as such it thrives in cool environments. Although the crop is most successful between the latitudes of 30° and 60°N and 27° and 40°S (Nuttonson, 1955), wheat can be grown beyond these limits, from within the Arctic Circle to higher elevations near the equator. Development research by the International Maize and Wheat Improvement Center (CIMMYT) during the past two decades (Saunders and Hettel, 1994) has shown that wheat production in much warmer areas is technologically feasible. In altitude, the crop is grown from sea level to more than 3000 masl (meters above sea level) and it has been reported at 4570 masl in Tibet. The minimum water content required in the grain for wheat germination is 35 to 45 percent by weight (Evans *et al*., 1975). The optimum growing temperature is about 25°C, with minimum and maximum growth temperatures of 3° to 4°C and 30° to 32°C, respectively (Briggle, 1980).

Wheat quality is influenced by both genotypic and environmental factors (Loffer and Busch, 1982). Farooq *et al*., (2001) noted significant ($p \leq 0.01$) effect of varieties and lines on chemical composition of wheat, especially on protein fractions. Micronutrients and undernourishment is the major cause of deficiencies in the world's population.

The world's population is increasing by one billion in every 11 years and at the present rate, it is expected to be 8.5 billion by the year 2025. Keeping pace with the increasing population and from the point of their future food security , sustained increase of wheat production and productivity is well understood in different corners of the world. Wheat grains are highly nutritive as they are rich in energy, carbohydrates, dietary fiber, fat, protein, thiamine, riboflavin, niacin, pantothenic acid, vitamin B6, folate, calcium, iron, magnesium, phosphorus, potassium, zinc and manganese. Due to the high nutritive value, wheat grains are eaten in various forms across cultures and continents.

Wheat crop is subjected to a number of diseases, which are responsible for plummeting overall production to a greater extent because the plant in all stages of growth and in all natural environments are subjected to various biotic and abiotic stresses that hamper with normal metabolism. Among the several constraints, for low productivity, the prevalence of number of pathogens infecting the crop is of supreme importance. Diseases are the major threat to wheat production and they are taking heavy toll of the crop in the country and elsewhere in the world. Basic and strategic research is required emphasizing major advancement in genetic yield potential,

durable resistance and genetic enhancement. Precision farming to enhance input use efficiency, optimal use of renewable resources and enhance the sustainability of wheat based cropping systems is necessary. Monitoring of emerging diseases under climate change scenario required to develop strategies to mitigate crop loss due to sudden spurt of pest and diseases.

Recent yield trials conducted by different breeding centres around the world have shown that the production of bread wheat (*Triticum aestivum* L.) is being constrained by several biotic and abiotic stresses (Dubin and van Ginkel, 1991; Hobbs and Giri, 1997; Dubin and Duveiller, 2000; Regmi *et al.,* 2002; Sharma and Duveiller, 2003; Duveiller, 2004). The warmer parts of the world like Latin America, Africa, Asia, Southern Asia etc., are mainly affected by *Bipolaris sorokiniana* a notorious wheat fungal pathogen (Dubin and van Ginkel, 1991). The pathogen acts as a causal agent for various diseases like head blight, seedling blight, foliar blight/ spot blotch, common root rot and black point of wheat, barley, other small cereal grains and grasses (Wiese, 1998). Among the all diseases, caused by this pathogen spot blotch of wheat is considered as one of the most important diseases in those mega environment which is characterized by high temperature and humidity (van Ginkel and Rajaram, 1993). However, it is also gradually instigating serious concerns among places with irrigated, low rainfall and temperate growing conditions (van Ginkel and Rajaram, 1993; Anonymous, 1995). Appearance of the disease is influenced by weather factors especially temperature and high relative humidity but details of the weather factors relation with disease has not been studied so far.

The disease is increasingly becoming a cause of concern particularly in the warm and humid environments of Indian sub-continent (Duveiller *et al.,* 2005) where the mean temperature of the coolest month is higher than 17.5˚C (Dubin *et al*., 1998). More recently, spot blotch has also expanded into the cooler, non-traditional irrigated rice-wheat growing areas (Duveiller and Gilchrist, 1994; Sharma *et al.,* 2007). Since high temperatures aggravate spot blotch severity (de Lespinay, 2004; Sharma and Duveiller, 2004; Duveiller *et al.,* 2005) wheat yield losses in the region could be due to increase in temperature which resulted in spot blotch epidemic over the recent years (Sharma *et al.,* 2007). Moreover, recent survey indicated that spot blotch spread to non-endemic areas in Pakistan (Shamim *et al.,* 2010) might be due to temperature rise. In Indian sub-continent wheat is already facing yield reduction due to terminal heat stress and increased spot blotch severity may aggravate further yield

damage (Nagarajan, 2005; Juroszek and von Tiedemann, 2013). Climate change in South Asia not only increased the temperature but increased number of cloudy and foggy days during winter months also (Debi, 2003). It reduced solar radiation and lengthened the duration of high relative humidity resulting in early establishment of spot blotch in wheat (Sharma *et al.,* 2007). Due to global climate change, mean global temperature rise is expected in the range 0.5-2.0°C by the end of this century (IPCC, 2013). This is likely to influence or change all the elements of a disease triangle i.e. host, pathogen and weather factors and their interactions (Anderson *et al.,* 2004; Burdon *et al.,* 2006; Legreve and Duveiller, 2010). Climate change in terms of elevated CO_2 and temperature is now a concern as the change may probably influence the occurrence, prevalence and severity of plant diseases as evidenced from number of observations, anticipated, or possible consequences on crop health worldwide (Chakraborty *et al*., 2008; Legreve and Duveiller, 2010). There are enough indication that climate change could alter stages and rates of development of the pathogen, modify host resistance, and results in changes in the physiology of host-pathogen interactions (Coakley and Schrem, 1996). Few studies indicated that spot blotch infection in wheat is highly influenced by weather factors mainly temperature and high relative humidity (Singh *et al.,* 1997; Singh *et al.,* 1998; Mehta, 1998; Reis, 1991; Senthil, 2004). Disease response in the field can vary differently because they experience a larger number and a wider range of environmental cues (including variations in light quality, temperature and RH level). However, little is known about the importance of small changes in temperature under field conditions, or the biometeorological basis of responses to such changes.

A perusal of literature has indicated that there is no systematic study to develop monitoring and forecasting for the disease although spot blotch infection is highly influenced by temperature and high RH or wetness or dew hours. Urgently there is a need of forecasting model for climate change impact assessment on spot blotch. Forecasting model is necessary for assessment of potential risk of spot blotch distribution and abundance for both strategic and tactical diseases management decision.

Therefore, following objectives have been contemplated:

- Development of spot blotch forecasting system based on infection favorable weather conditions as well as rate of incubation period completion.

- Determination of elevated CO_2 and temperature level on spot blotch infection for the assessment of climate change impact.
- Mapping of spot blotch infection distribution and abundance throughout Indo-Gangetic plains under climate change scenario based on favorable conditions and developmental rate.

CHAPTER-II BACKGROUND

Spot Blotch Disease

Several biotic and abiotic factors are the stumbling blocks in realizing the potential yield of wheat. Among the biotic factors, the most widely prevalent, notorious and shifty enemy on wheat is spot blotch disease caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker syn. *Drechslera sorokiniana* (Sacc.) Subrm and Jain (syn. *Helminthosporium sativum* Pamm., King & Bakke), teleomorph *Cochliobolus sativus* (Ito & Kuribayashi) Drechsl. ex Dastur (Dubin and van Ginkel, 1991; Joshi *et al.*, 2004a,b; Sharma and Duveiller, 2004; Pandey *et al.*, 2005).

Classification of the Pathogen

Phylum	:	Ascomycota
Class	:	Loculoascomycetes
Order	:	Pleosporales
Family	:	Pleosporaceae
Genus	:	*Cochliobolus*
Species	:	*sativus*

The genus *Cochliobolus* produces globose ascomata with a long cylindrical neck, obclavate cylindrical asci, and helically coiled filiform ascospores. Mycelium of *B. sorokiniana* is olive-brown and it produces light grey colonies at early stage of growth in potato dextrose agar medium, later turns into black to olivaceous black. Conidiophores are 6-10 × 110-220 µm, brown, erect, unbranched, single or clustered, septate. Conidia are 15-28 × 40-120 µm, straight to slightly curved, oblong, fusiform to broadly ellipsoid, olive brown to dark brown, tapered towards the end and have a prominent basal scar, smooth walled and having 3- 10 thick walled transverse septa (Mathre, 1987). In the host tissue, it produces septate mycelium, which ramifies both inter- and intracellularly. Conidiophores are long, septate, simple, dark brown to olivaceous at the base and somewhat paler at the growing tip. They arise in tufts through stomata, ruptured epidermis or wounds and produce conidia successively on new growing points. The points of attachment of successive conidia are marked by scars at the regular intervals on the conidiophores. The perfect stage i.e., the ascigenous state was observed in the laboratory on natural media in the presence of

opposite mating types, and was first described as *Ophiobolus sativus*. It was later renamed *C. sativus* (Ito & Kuribayashi) Drechsler ex Dastur, 1942 (Dastur, 1942).

Distribution and Economy of Spot blotch

Spot blotch is widely prevalent in warm and humid environments. The pathogen is distributed worldwide, across South East Asia (Saari, 1998), North and Latin America, Africa (Duczek and Jones-Flors, 1993), India (Joshi *et al.*, 2002), China (Chang and Wn, 1998) and Brazil (Mehta, 1998). Globally, an estimated 25 million hectares of wheat growing areas are affected by the disease (Duveiller *et al.*, 2005, Van Ginkel and Rajaram, 1998). About 10 million hectares of wheat growing belt in Indian subcontinent is affected by the disease, out of which 9 million hectares belonging to Indo-Gangetic plains predominantly with rice-wheat cropping system shared the maximum (Nagarajan and Kumar, 1998; Ruckstuhl, 1998; Singh *et al.*, 1998; Shamim *et al.*, 2010).

Yield Loss

The destructive capacity of this pathogen is evident from the reports around the world. Grain yield reductions due to spot blotch are variable but are of great significance in warmer areas of South Asia (Saari, 1998; Sharma and Duveiller, 2004). On an average, a South Asian country loses 20% of crop yield through leaf blight disease (Saari, 1998). The spot blotch imparts yield loss in the tune of 20-80% and the extent of loss depends upon the severity of occurrence (Saari, 1998; Duveiller and Sharma, 2009). Yield loss was estimated to be 18-22% in India (Singh *et al.*, 1997), which can be devastating for farmers in the Eastern Gangetic Plains, who frequently have small holdings with little land or profitability (Joshi *et al.*, 2007). In Nepal, under rice-wheat cropping system, spot blight severity went up to 100% and 70% in 2004 and 2005 respectively (Sharma and Duveiller, 2007). Spot blotch has been reported to cause15% grain yield reduction in Bangladesh (Alam *et al.*, 1998) and China (Xiao *et al.*, 1998). The pathogen also causes grain yield losses up to 10, 15 and 20% through common root rot and seedling blight in countries like Scotland, Canada, Brazil etc. (Murray *et al.*, 1998). Economic yield losses due to spot blotch was estimated 3-20 % in India, 71% in Bangladesh, 20-30% (but sometimes above 75%) in China, 16.2-29% in Nepal and 40% in Philippines (Senthil, 2004).

Disease symptoms

Symptoms mainly develop on sub-crown internodes, stem, leaves, awns, glumes and seeds. The main symptom caused by the pathogen is spot blotch. The early lesions on leaves are 1-2 mm long, small and dark brown in colour. There is no sign of chlorotic margin at the initial stage of infection. In the later stage in case of a susceptible genotype the small lesions extends very rapidly in oval to elongated blotches, light brown to dark brown in colour. They may reach several centimeters before coalescing and inducing the death of the leaf. Fruiting structures develop readily under humid conditions and are generally easily observed on old lesions. If spikelets are affected, it can result in shrivelled grain and black point, a dark staining of the embryo end of the seed (Dubin and Duveiller, 2000).

Diseased seedlings develop dark brown lesion on the coleoptiles, crowns, stems and roots. Death of the seedlings may occur before or soon after emergence. Common root rot is distinguished by dark brown to black necrotic lesion on roots, subcrown, internodes and basal portion of the stem. At severity, multiple lesions often coalesce to form large areas of necrosis (Mathre, 1987). Plants with common root rot produce fewer tillers and fewer kernels per panicle.

Epidemiology

Foliar blight development and severity of the disease is directly related to the minimum tillage or surface seeding, irrigation, low soil fertility, sowing density, crop growth stage, late rain during crop cycle, heat stress during grain filling as a result of late planting, high temperature in the field and relative humidity favouring long duration (>12 hours) of leaf wetness (Nema and Joshi, 1973; da Luz and Bergstrom, 1986; Saunders, 1988; Reis, 1991; Sentelhas *et al.*, 1993; Sharma and Duveiller, 2003; Duveiller, 2004; Duveiller *et al.*, 2005, Bailey *et al*., 2000). Zadoks growth stage 60 was used to assess the disease severity and inter-relationship among tan spot and spot blotch (Wegulo *et al*., 2009). Even at the end of the monsoon and in absence of rainfall, high relative humidity arising from high levels of soil residual moisture along with foggy days allows long hours of wetness on leaf blades that can last until late January in Indo-Gangetic Plains, creating ideal conditions for the establishment and multiplication of wheat pathogen (White and Rodriguez-Aguilar, 2001; de Lespiny, 2004). In Brazil, Reis (1991) suggested that, for foliar blight outbreaks to occur, wheat leaves must remain wet for >18 h at a mean temperature of 18˚C or higher. Moderate to warm temperatures (18˚C to 32˚C) favours the growth of *B.*

sorokiniana. In Asia, Nema and Joshi (1973) and Singh *et al.*, (1998) reported that infection was more rapid and more severe at 28°C than at lower temperatures. Area under disease progress curve values (AUDPC), conducted during 26th November 2002 to 26th December 2003 for calculating the epidemiological study of disease development, increased significantly as a function of sowing time (Duveiller *et al*., 2005). The higher values of AUDPC/day or AUDPC/degree day under late-sown conditions are most likely caused by heat stress, which enhanced HLB development (Nema and Joshi, 1973; Sharma and Duveiller, 2003). Delayed seeding for wheat, grown after rice in eastern India and Nepal also results in higher losses of grain yield and total kernel weight due to foliar blight (Hobbs and Giri, 1997; Singh *et al.*, 1998; Duveiller *et al.*, 2005).

Factors affecting disease development

Disease severity is also dependent on the method of inoculation. An inoculation apparatus was developed by Robinson and Hodges (1976) for the evaluation of *B. sorokininana* lesion on progressively older leaves of *Poa prantesis.* These lesion numbers were based on leaf area measurements, and different lesion types were used to quantify the disease severity. Aggressiveness of the pathogen is also dependent on the concentration of the inoculums (Liatukas and Ruzgas, 2012). A correlation was developed by banding pattern of RAPD for morphological, aggressiveness and genetic variations of *B. sorokiniana* (Iram and Ahmed, 2004). *In vitro* survival ability of the pathogen was studied by Malaker *et al.,* (2007) in soil and wheat residue. It was found that the pathogen could survive for 12 months in free residue stored at room temperature. Another study was carried out by Chand *et al.,* (2003) to find out the extent of variability and the probable cause of its emergence in natural populations of spot blotch pathogen *B.sorokiniana* of wheat. Pathogenicity studies on a global collection of *B.sorokiniana* monoconidial strains on a differential set of wheat entries showed that differences between strains existed but no clear host specialization was found (Hetzler *et al*., 1991). Similarly, Ruckstuhl (1998) found differences between groups of strains from South Asia, Mexico and Bolivia based on random amplified polymorphic DNA (RAPD) analysis but without any relationship to pathogenicity. These studies further support the data that *B. sorokiniana* forms a continuum of strains differing in aggressiveness but without clear physiological specialization (Maraite *et al*., 1998).

Disease Cycle

B. sorokiniana is a saprophyte and survives primarily as thick walled conidia. The sexual stage is not important in the disease cycle. The pathogen perennates both externally as conidia and internally as mycelium in the seeds, as well as in infected crop residues, volunteer plants, secondary hosts and free dormant conidia in the soil (Reis, 1991). However, the role of infected seed as a primary source of inoculums appears to be important and it is the main source of inoculums of leaf blight pathogens. Along the germination of the diseased seeds, the perennating organs of the causal organism become active. This is the starting point of the disease. It germinates completely in four hours, and then appressoria forms at the juncture of epidermal cell wall after eight hours and hyphae from initially infected cells enter adjacent cells in 24 hours, which results in the granularisation of the host cytoplasm. Then fungus is transmitted to the plumules and coleoptiles tips with an efficiency reaching upto 87% (Reis and Forcelini, 1993). Maximum development of symptoms appears when the leaves remain wet for more than 18 hours with a mean temperature greater than 18˚C (Couture and Sutton, 1978). Under favourable conditions, hypha produces conidiophores, which emerge out through stomata of the host tissue. The emerging conidiophores produce a succession of conidia, which are transmitted by rain splashes and wind, thus building up polycyclic epidemics. Conidia on germination produce germ tube, which is surrounded by thick mucilaginous substrata. This mucilaginous substratum enables the germinating conidia to remain adhered to the host surface. The germ tube then swells to produce appresorium from which infection hyphae are developed. The infection hyphae then enter the host tissue either through stomata or by rupturing through epidermis. Immediately after the entrance in the host tissue, the infection hypha divides rapidly and ramifies along the intercellular spaces of the mesophyll tissue (Acharya *et al.,* 2011).

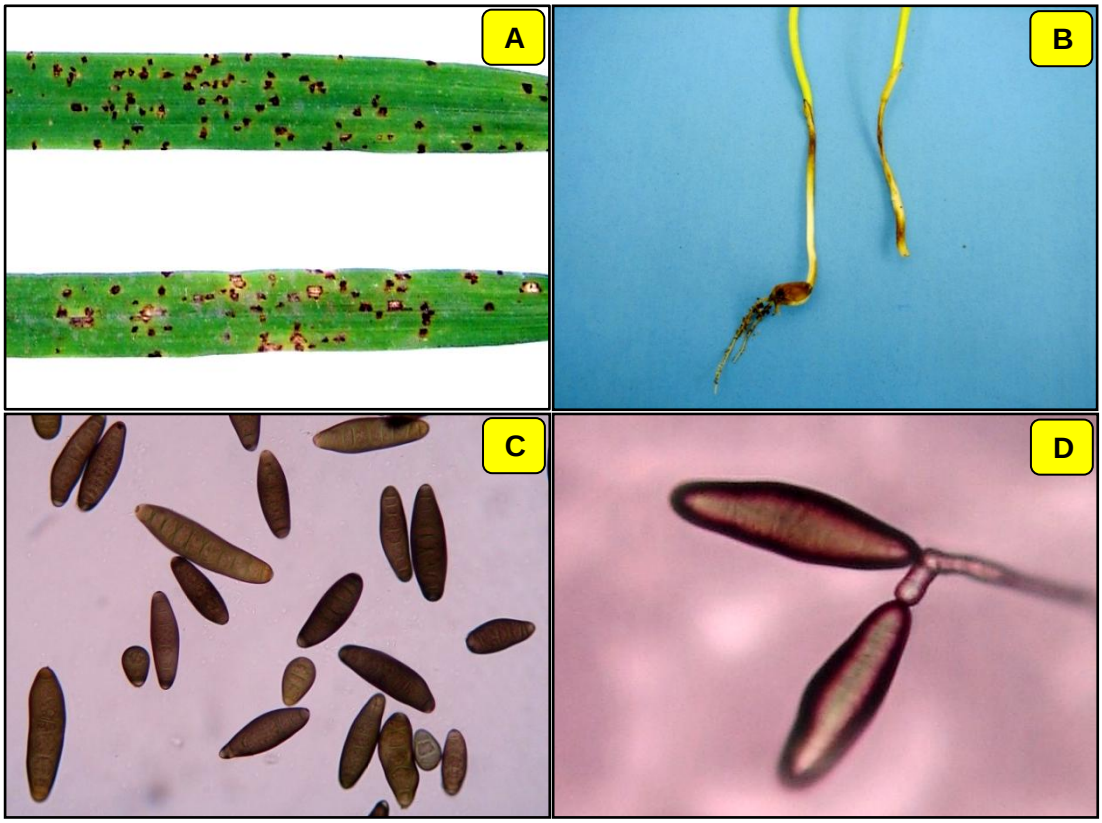


Fig. 2.1: Symptoms of spot blotch disease in wheat. Spots on leaves (A) and collar rot on seedlings (B), conidia (C) and conidiophore (D) of *Bipolaris sorokiniana*.

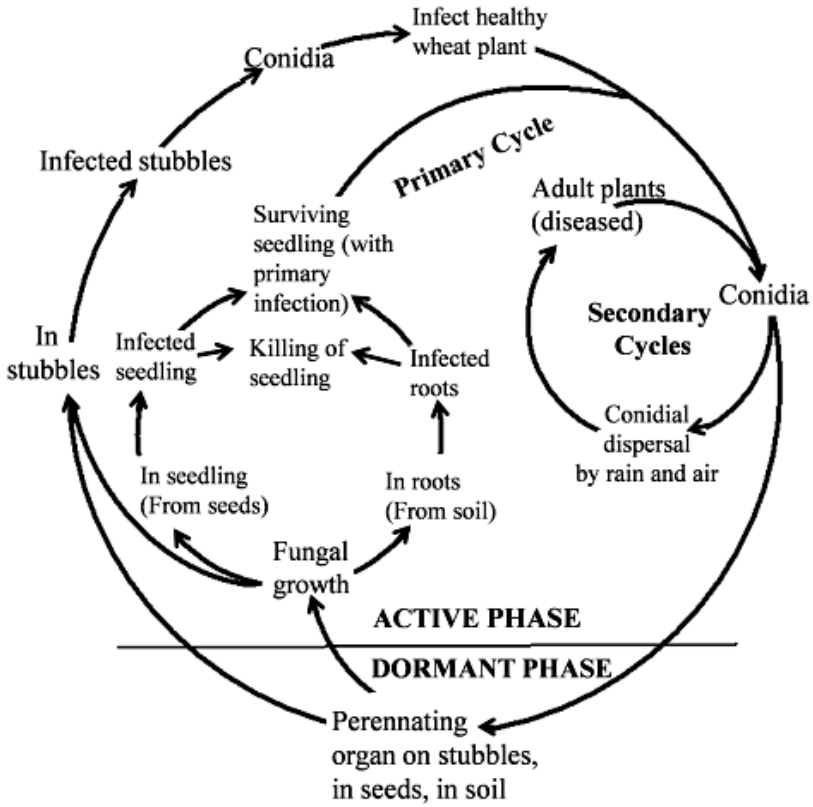


Fig. 2.2: Disease cycle of spot blotch caused by *Bipolaris sorokiniana* (Acharya *et al.*, 2011).

Strategies for management of spot blotch disease

On a worldwide scale, yield losses in wheat and barley caused by *B. sorokiniana* indicate the need to search for alternative strategies for disease control. Disease incidents can be controlled in a number of ways. Integrated pest management is by far the best method of controlling the pathogen (Mehta, 1993; Dubin and Duveiller, 2000). Such program integrates the use of: (i) cultural practice, (ii) crop rotation, (iii) seed treatment, (iv) biological control, (v) foliar fungicide and (vi) disease resistant varieties.

Biological control

Natural resistance of wheat towards this pathogen is found to be low (Aggarwal *et al.,* 2004). However, there is a possibility of biological control of spot blotch disease (Mandal *et al.,* 1999). Instead, biological protection strategies look more promising. Pretreatment of wheat leaves with the inappropriate *B. oryzae* (inducer organism) reduced spot blotch lesion numbers and sizes of *B. sorokiniana* on the same leaves (Sarhan *et al.,* 1991). A preliminary study identified antifungal substances in apoplastic fluids of 'induced' plants that were able to prevent spore germination of *B. sorokiniana in vitro* (Chakraborty and Sinha, 1984). Reduced symptoms could also be achieved, although not to the full extent, after spraying with the bacterial biocontrol agent *Pseudomonas chlororaphis*, strain MA 342. Treated seeds could be stored dry for at least 2 years without losing the disease suppressing effect of the bacterial treatment (Johnsson *et al.,* 1998). Suppression of soil-borne fungi, including *B. sorokiniana*, has also been observed in the presence of isothiocyanates released into soil by *Brassica* species (Kirkegaard *et al.,* 1996). The saprophytic ascomycete, *Chaetomium globosum Kunze*, is a potential antagonist of several soil and seed borne plant pathogens (Vannacci and Harman, 1987; Walther and Gindrat, 1988; di Pierto, 1990) and recent studies have emphasized the role of *C. globosum* in controlling spot blotch of wheat caused by *C. sativus* (Biswas *et al.,* 2000). A thorough study made by Aggarwal *et al*., (2004), has highlighted the potential antagonism of an antifungal metabolite produced by *C. globosum* against *C. sativus* both *in vitro* and *in vivo* conditions.

Cultural practice

Sanitation is usually reserved for the physical measures that are used to eliminate sources of inoculums. Clearing or plugging the stubble, grass weeds and volunteer cereals reduce inoculums of *B. sorokiniana* (Diehl *et al.,* 1982). Selection of disease

free seeds and utilizing proper fertilization technique can also be effectively controlled spot blotch severity. In Brazil, it is recommended not to plant seed lots with more than 30% black point in order to limit spot blotch incidence (Reis, 1991). Macro and micronutrients have long been recognized as being associated with size, quality and yield of crops. Besides increasing grain quality, the main objective of nutrient application is also to protect crops against the pathogen. Macro and micronutrients helps plants to withstand pathogen attack at the time of stressful conditions and also provides advantage of plants and disadvantage to the pathogen (Palti, 1981). Good crop husbandry and optimum agronomy may also reduce spot blotch disease severity up to certain level (Sharma *et al.,* 2006). In this connection limited previous studies were available to indicate the role of potash in reducing spot blotch severity (Krupinsky and Tanaka, 2000; Regmi *et al*., 2002; Duveiller, 2004). Later during the year 2001 and 2002 a field study conducted in Rampur, Nepal, using two wheat varieties (Bhrikuti and Sonalika) to determine the effect of nitrogen, phosphorus, and potassium fertilization on reducing spot blotch severity. Results of this field study showed that the balanced application of nitrogen, phosphorous and potassium reduced disease severity by 15 and 22% respectively (Sharma *et al.,* 2006). Potassium helps to prevent disease development by hindering multiplication, development and survival of pathogen and controlling the internal metabolism of the plant and thus affecting food supply for the pathogen, as well as preventing the establishment of the pathogen and its spread within the plant (Perrenoud, 1990).

Crop rotations

Crop rotation favours beneficial soil organisms as well as promotes better plant nutrition. According to Reis *et al.,* (1998) eradicant fungicide treatment and crop rotation with non-host crops can control spot blotch. As this pathogen has a wide host range, so there are some difficulties to find out the suitable non-host crop. According to the report of Iftikhar *et al.,* (2009) 11 plants including *Avena sativa, Brassica campestris, Glycine max, Hordeum vulgare, Lens culinaris, Pennisetum amaricanum, Sesamum indicum, Sorghum bicolour, Vigna radiata, Vigna mungo* and *Zea mays* has already been reported to be the host of this pathogen. More than 29 species of poaceae and other crops in North-eastern China, 65 species of poaceae in Yellow and hai river region and 17 plant species in Guandong province also serves as the host of *B. sorokiniana* (Chang and Wn, 1998). Though some scientists

suggests that rotating crops with rape, sorghum and soybean might reduce the carry over population of the pathogen to very low level, selection of suitable non-host crop requires further research.

Seed treatment and the use of fungicide

Seeds are one of the important sources of primary infection. Therefore, seed treatment with a suitable fungicide reduces the carry over inoculums potential, but unless soil inoculums are reduced, seed treatments alone offer no benefit. Although various kinds of systemic fungicides are now available for seed treatment, the decision to treat the seed should be carefully considered. As determined by the blotter test, seed treatment should not be applied if the seed infection level is less than 20% and germination is within standards. Seed lots with less than 20% infection should be treated only if percent germination is lower than the standard and there is shortage of seed (Mehta, 1998). In Brazil, seed treatment is recommended based on seed health analysis, soil condition and crop rotation (Reis, 1991). Seed treatment with suitable fungicides such as Captan, Mancozeb, Maneb, Thiram, Pentachloronitrobenzene (PCNB) or Carboxin guazatine plus, Iprodione and Triadimefon are useful in protecting germinating seeds and seedlings from seedling blights (Stack and McMullen, 1988; Mehta, 1993). Sharma *et al.,* (2005) has reported that seed treatment with Vitavax 200B and Carbendazine improves early plant establishment in heavy soil predominating areas where wheat is cultivated after rice.

Foliar fungicides

Use of fungicides has proven useful and economical in the control of spot blotch (Viedma and Kohli, 1998). The Triazole group (e.g. Tebuconazole and Propinazole) especially have proven to be very effective against spot blotch disease. The use of fungicide Opus (Epoxiconazole) reduced disease severity to below 10%, whichsuggest its value in controlling spot blotch resistant wheat cultivars (Duveiller *et al.,* 2005; Sharma *et al.,* 2005). Later study conducted by Sharma and Duveiller (2006), shows that the fungicide Opus effectively controls spot blotch disease under soil nutrient stressed farmers'field conditions. However, questions have been raised regarding their use and environmental sustainability (Aggarwal *et al.,* 2004).

Disease resistant varieties

Cultivation of disease resistant varieties is an economical and effective option for controlling any plant disease. However, conventional breeding of wheat for selection

of genotypes resistant for spot blotch has made very limited progress in the past (Sharma *et al.,* 2007). A few studies from South Asia have reported only low to moderate success in breeding for spot blotch and foliar blight resistance (Alam *et al.,* 1994; Devkota, 1994; Bhandari *et al.,* 2003; Sharma *et al.,* 2004; Siddique *et al.,* 2006). Eventhough, the resistant cultivars of South Asia e.g.,"Gautam" have been reported to suffer from substantial grain yield loss under severe epidemic of spot blotch (Duveiller *et al.,* 2005). To date, the best sources of resistance were discovered in the Brazilian and Zambian wheat lines (Rajaram, 1988; Dubin and van Ginkel, 1991). A few Chinese wheat genotypes like SW895422, Chirya1, Chirya3, Chirya7, NL781, and NL785 also showed significant resistance levels to spot blotch (Kohli *et al.,* 1991). *Triticum* shows different levels of spot blotch resistance, between species with same and different ploidy and genomes. High frequency of resistance was noted in *T. timopheevii, T. araricum, T. boeoticum, T. persicumand T. urartu* widespread in Transcauc asia as well as in T. Sphaerococcum which is native to Indian peninsula (Smurova and Mikhailova, 2007). Somaclonal variation is regarded as a supplementary tool to the well-established breeding approaches (Cheng *et al.,* 1992; Karp, 1995; Ivanov *et al.,* 1998). In wheat, somaclonal variants have been reported for various plant traits. Somaclones were regenerated by Arun *et al.,* (2003) from immature embryos of two spring wheat varieties HUW-206 and HUW-234 displayed improved earliness, enhanced resistance to spot blotch disease and increased yield over parents established in regeneration.

Climate change and its effects on plant diseases

The dynamic of disease, impact and geographic distribution of the pathogen, plant physiology, host-plant resistance is/are also affected by the climatic change. Recent environmental changes such as shortage in rainfall, extended hot and dry climates and extreme weather events in large rural communities affecting agricultural production, result in frequent grower diseases and pest epidemics, appearance of peculiar symptoms such as plant decline and death, and sudden diffusion of particular pests. It is hypothesized that climate change will increase susceptibility to Spot blotch because of increased abiotic stress and the necrotrophic lifestyle of the fungus.

Climate change in terms of elevated CO_2 and temperature is now a concern as the change may probably influence the occurrence, prevalence and severity of plant diseases as evidenced from number of observations, anticipated, or possible

consequences on crop health worldwide (Chakraborty *et al*., 2008, Legreve and Duveiller, 2010). Changes in climate may modulate host susceptibility/resistance responses to pathogens (van Maanen and Xu, 2003). Increasing trend in temperature during winter probably makes favourable situation for spot blotch in north western part. Under climate change scenario uncertainties about disease occurrence prevails. Hence, there is a need to anticipate invasion risks from both exotic and indigenous pests.

Due to global climate change, mean global temperature rise is expected in the range 0.5-2.0°C by the end of this century (IPCC, 2013). This is likely to influence or change all the elements of a disease triangle i.e. host, pathogen and weather factors and their interactions (Anderson *et al.,* 2004; Burdon *et al.,* 2006; Legreve and Duveiller, 2010). Effect of changing environment on the pathogen characteristics such as frequency of generations and predictions on how changes in temperature are likely to influence disease severity are largely unknown. There are enough indication that climate change could alter stages and rates of development of the pathogen, modify host resistance, and results in changes in the physiology of host-pathogen interactions (Coakley and Schrem, 1996). This may also affect disease epidemics; alter spatial distribution over agro-ecological zones, habitats and distribution patterns of plant diseases (Chakraborty and Newton, 2011). Temperature rise has been predicted in the range 1.2-2.8°C in Indo-Gangetic plains and rainfall is expected to increase about 20% by the end of century (Kothawale *et al.,* 2010). A rise of temperature in the range of 0.5-0.8°C above pre-industrial era is already reported (Rupa-Kumar *et al.,* 2006; Kothawale *et al.,* 2010; IPCC, 2013). Garrett *et al.,* (2006) has underlined the intricate interrelationships between plant disease and climate. Climate change is characterized by greater increases in minimum temperature than maximum temperature and therefore, improved understanding of the temperature response is needed to better quantify and reduce uncertainties in climate change impact assessments (Lobell and Ortiz-Monasterio, 2007).

Impact of elevated CO_2 on physiology of plants

Anthropogenic intensification leads to the increase in carbon dioxide and other green house gases. The increase in atmospheric CO_2 derived from the combustion of fossil fuels and changes in land use (Forster *et al.,* 2007). According to the Intergovernmental Panel on Climate Change (IPCC), the pre-industrial levels of carbon in the atmosphere rose from 285 μmol l^{-1} (600 gigatonnes (Gt) to the current

level of 384 μmol l^{-1} (800 Gt) and the predicted rise in the atmospheric CO_2 would approach 1000 Gt by the year 2050. Such an abnormal rise in the levels of atmospheric CO_2 would result in direct and indirect global climate changes.

Climate change affects plant growth and development primarily due to changes in photosynthetic carbon assimilation patterns. Carbon Dioxide functions as a additional signal molecule in synchronizing metabolic processes especially related to photosynthesis in leaves (Luttge, 2007). The acclimatory responses of plants to the rapidly changing environment includes accelerates the rate of plant photosynthesis, often causing an increase in plant productivity (Drake *et al*., 1997; Ainsworth *et al.,* 2002; Long *et al*., 2004) and understanding the potential impacts of multiple interacting factors (water availability, temperature, soil nutrition and ozone) . Growth in elevated CO_2 generally increases the carbon- nitrogen ratio (C:N) of plant tissues (Heagle *et al*., 1998; Rogers *et al*., 2004; Hamilton *et al*., 2005), reducing the nutritional quality for protein-limited insects (Coviella and Trumble, 1999).

On elevated CO_2, two main driving forces influence pathogen and disease development during. In the short term, disease severity levels are determined by the opposing effects of enhanced host resistance that slows host invasion versus enlarged plant canopy that offers more infection sites and produces a microclimate conducive to disease development (Chakraborty, 2005). More importantly, in the long term, higher pathogen fecundity, inoculum trapping by an enlarged plant canopy and a higher number of infection cycles interact with the effects of enhanced host plant resistance to determine host–pathogen adaptation (Chakraborty and Datta, 2003).

For *in vitro* experimentation, a number of technologies have been developed to study the impact of rising CO_2 in agriculture system. Phytotrons, FACE (Free Air CO_2 enrichment), OTCs (Open Top Chambers) and SPAR (Soil Plant Atmosphere Research) is being currently used for crop response studies (Uprety *et al*., 2006). Taylor *et al*., (2001) studied the response of hybrid poplar to elevated CO_2 in contrasting growth environments such as controlled environment chamber (CE), open-top chamber (OTC) and poplar free air CO_2 enrichment (POPFACE) and concluded that enhanced leaf area development was a consistent response to elevated CO_2, but declined in magnitude with time. Similarly, the transcriptome responses of rapidly growing and fully expanded leaves of *Glycine max* to elevated CO_2 in SOYFACE facility were investigated using cDNA microarrays. Clustering of the identified transcripts showed that transcripts involved in cell growth and cell

proliferation were more highly expressed in growing leaves that developed at elevated CO_2 compared to growing leaves that developed at ambient CO_2 (Ainsworth *et al.,* 2006). For data analysis, several models viz. CERES-Wheat, are also developed for the prediction of biomass production and yield under a variety of growth environments in both field and closed chamber conditions (Tubiello *et al*., 1999).

Since CO_2 enrichment is not a stress for plants, plants might have lacked the need to acclimate to elevating CO_2. The responses described here are attributed to secondary responses related to excess carbohydrate accumulation or decreased N content rather than direct responses to CO_2. It may leads to increase in plant mass. The suppression of photosynthesis by CO_2 enrichment is always associated with decreases in leaf N and Rubisco contents. Leaf senescence and plant development are also accelerated by CO_2 enrichment. However, they are independent of each other in some species (Makino and Mae, 1999). CO_2 enrichment also enhanced wheat grain yield mainly through increasing the grain number, and, consequently, the harvest index (Wood *et al.,* 2004).

Stomatal morphology, distribution and behaviour respond to a spectrum of signals, from intracellular signalling to global climatic change (Hethrington and Woodward, 2003). Crop plants have among the largest reductions in stomatal conductance at elevated CO_2, thereby could reduce the water use by vegetation. This reduction in stomatal conductance may vary considerably with light, temperature and humidity (Bunce, 2004). Miller-Rushing *et al.,* (2009) reported declined stomatal density and increased guard cell length over last 100 years in changed environmental conditions. Lower stomatal conductance was observed at elevated CO_2 (757 μmol mol^{-1}) in spring wheat (*Triticum aestivum* L. cv. Alcalá) grown in field conditions under temperature gradient tunnels (Del Pozo *et al*., 2005). Similarly, elevated CO_2 concentration brought reduction in stomatal conductance and increase in stomatal index, size of stomatal cell guard, stroma and epidermal cell. Such acclimatization helps in regulation of photosynthesis (Uprety *et al*., 2002).

Effect of climate change on spot blotch disease

Few studies indicated that spot blotch infection in wheat is highly influenced by weather factors mainly temperature and high relative humidity (Singh *et al.,* 1997; Singh *et al.,* 1998; Mehta, 1998; Reis, 1991; Senthil, 2004). Disease response in the field can vary differently because they experience a larger number and a wider range

of environmental cues (including variations in light quality, temperature and RH level). However, little is known about the importance of small changes in temperature under field conditions, or the biometeorological basis of responses to such changes.

The disease is increasingly becoming a cause of concern particularly in the warm and humid environments of Indian sub-continent (Duveiller *et al.,* 2005) where the mean temperature of the coolest month is higher than 17.5˚C (Dubin *et al.,* 1998). More recently, spot blotch has also expanded into the cooler, non-traditional irrigated rice-wheat growing areas (Duveiller and Gilchrist, 1994; Sharma *et al.,* 2007). Since high temperatures aggravate spot blotch severity (de Lespinay, 2004; Sharma and Duveiller, 2004; Duveiller *et al.,* 2005) wheat yield losses in the region could be due to increase in temperature which resulted in spot blotch epidemic over the recent years (Sharma *et al.,* 2007). Moreover, recent survey indicated that spot blotch spread to non-endemic areas in Pakistan (Shamim *et al.,* 2010) might be due to temperature rise. Recently, epidemics of spot blotch in wheat have increased in many parts of Nepal. This trend may be due to changes in climate, cultivation practices, or pathogen populations (Mahto *et al.*, 2011; Ortiz *et al.,* 2008). In Indian sub-continent wheat is already facing yield reduction due to terminal heat stress and increased spot blotch severity may aggravate further yield damage (Nagarajan, 2005; Juroszek and von Tiedemann, 2013). Climate change in South Asia not only increased the temperature but increased number of cloudy and foggy days during winter months also (Debi, 2003). It reduced solar radiation and lengthened the duration of high relative humidity resulting in early establishment of spot blotch in wheat (Sharma *et al.*, 2007).

Prediction/Forecasting models

The CLIMEX software package was developed to predict the potential geographic range of an organism based on its climatic requirements (Sutherst and Maywald, 1985). It has most often been used for invasive plants and insects, and their insect biological control agents (Robertson *et al.,* 2008; Ulrichs and Hopper, 2008; Bower *et al.,* 2009; Sutherst and Bourne, 2009). However, CLIMEX also has been used to accurately predict the distribution of pathogens detrimental to agriculture and forestry (Pivona and Yang, 2004; Yonow *et al.,* 2004; Viljanen-Rollinson *et al.,* 2006), to assess the potential distribution of an introduced rust fungus for biological

control of a wildland weed (Wood *et al.,* 2004), and to determine where to look for climatically adapted isolates in their native range (Scott *et al.,* 2002).

To produce risk prediction maps, CLIMEX model (Sutherst *et al.,* 2004) based on long-term climate data and downscaled general circulation models (GCMs) for global weather forecast have been used to assess the impacts of potential global climate change for diseases (Pivona and Yang, 2004; Salinari *et al.,* 2006). However, all these models do not provide specific weather information at the canopy level and thus provide only very gross estimates of conditions that affect plant and disease development. Hourly and daily estimates of weather data only could explain pathogen infection at canopy level and best suited for disease risk prediction (Gleason *et al.,* 1997). Therefore, temperature rise impact on disease, based on pathogen infection at canopy level using hourly resolution weather data is more realistic than long term weather based estimation of disease risk.

CLIMEX ^{TM} is a popular process oriented model which has been widely used to determine the potential distribution of poikilothermal organisms. Since Sherm and Yang (1999) pioneered the use of CLIMEX as a means of establishing the potential risk of invasion from a plant disease, relatively few additional studies have modelled pathogen distribution (Brasier and Scott, 1994; Desprez-Loustau *et al.,* 2007; Fisher *et al.,* 2011; Paul *et al.,* 2005; Watt *et al.,* 2009; Yonow *et al.,* 2004)

Change in favorable conditions in relation to rise in temperature may be useful to assess the impact of climate change on the disease. Temperature induced incubation and /or latent period models could describe developmental or generation rate of the pathogen on host (Madden *et al.,* 2007) as incubation period is generally characterized by a decrease in duration as temperature rises from the minimum to the optimum, then an increasing duration with higher temperatures (Analytis, 1977; Logan *et al.,* 1976; Pfender, 2001). Effect of changing environment on the pathogen characteristics such as frequency of generations and predictions on how changes in temperature will affect plant health requires knowledge on already observed effects of climate change on plant diseases, extrapolation from expert knowledge and experimental studies, and computer models (Garrett *et al.,* 2006). Evans *et al.,* (1992) developed a polynomial model for describing the effect of temperature and wetness duration on the severity of *Alternaria* leaf blight of muskmelon. Also the influence of temperature and hours of leaf wetness on brown patch severity in perennial ryegrass described by a polynomial regression model as:

$\text{Log}_{10}(Y+1) = b_0+b_1T+b_2T^2+b_3W+b_4W^2+b_5TW+b_6T^2W$ (Gross *et al.,* 1998). Latent period completion rate for stem rust in tollgrass and fescue was found to increase gradually from T_{min} up to T_{opt} and then decline more rapidly to reach zero at the T_{max} or lethal temperature (Pfender, 2001). Therefore, effect of diurnal temperature fluctuation either below or above these threshold levels ($T_{min,}$ T_{max} and T_{opt}) could be related with incubation or latent period to reflect the development of the pathogen on host. Such temperature-based developmental rate model is also likely to facilitate temperature rise effect on diseases that may happen under climate change regime. Disease distribution models are powerful tools to predict future disease epidemics, and provide support for developing strategies against new threats might occur. Distribution models predict potential geographical range for a disease based on two types of georeferenced data, biological data describing the species' known distribution (presence and absence) and meteorological data which describe the landscape conditions where the species is found (Paul *et al.,* 2005). Because of their reliance on climatic or weather data, these models are well suited to studies of the effects of climate change on plant disease, and exotic pest introductions. The current spot blotch scenario suggests climate change might have some role.

Zearfoss *et al.,* (2011) used a degree-day model for the latent period of *Stagonospora nodorum* blotch in winter wheat for describing the increase in number of lesions with pycnidia over accumulated thermal time. Molitor *et al.,* (2012) developed a cumulative degree-day-based model to calculate the duration of the incubation period of *Guignardia bidwellii* on leaves and clusters of *Vitis vinifera*. A temperature-based disease prediction model was developed in combination with geographical information systems (GIS)-linked climate databases for predicting geographic variation in Swiss Needle Cast disease severity on Douglas-fir to estimate disease levels across a portion of the Oregon Coast Range (Manter *et al.,* 2005). Pfender (2001) established a temperature-based model for latent-period duration in stem rust of perennial ryegrass and tall fescue. Percentage of one latent period completed per half hour (half-hourly rate), for perennial ryegrass was modeled as $(0.0156T–0.0206)\{1–\exp[0.497(T–35.5)]\}$, where T=average temperature (°C) during the half-hour period. For tall fescue the modeled rate was $(0.0109T–0.00214)\{1 – \exp[0.417(T– 35.5)]\}$. Latent-period duration (LP_{50}) is affected by the temperature imposed during latency. The response is generally characterized by a decrease in duration as temperature rises from the minimum to the optimum, then an

increasing duration with higher temperatures. Teng *et al.*, (1980) described the data for *P. hordei* on barley as $LP_{50}=19.7 - 0.085T^2 + 0.0025T^3$. For alfalfa rust, a linear decrease in logarithm (LP_{50}) with temperature from 14 to 30°C was observed (Webb and Nutter, 1997). Analytis (1977) developed a more flexible model that can more closely approximate a diversity of temperature response curves. This model produces an equation for relative rate that can conveniently compare different fungi, and requires transforming temperature to a proportion of the range between lower and upper limiting temperatures (0 to 1 scale) and growth rate to a 0 to 100% scale (percent of maximum rate).

A perusal of literature has indicated that there was no systematic study to establish any such models for forecasting spot blotch in wheat and simulate the impact of climate change on the disease in the region.

All investigations were conducted at the laboratory, net house and experimental field of Plant Pathology Division, Phytotron, and experimental field of Indian Agricultural Research Institute (28°38'23"N, 77°09'27"E, 228.61 m above mean sea-level), New Delhi, India.

3.1. Laboratory experiments

3.1.1 Preparation of inoculum

B. sorokiniana was isolated from one heavily infected field of Institute's experimental farm and its pathogenicity was proved comparing similarity with a pathogenic isolate of Indian Type Culture Collection. For sporulation, the fungus was grown 10 days in water agar at 24°C. The spore suspension was prepared and adjusted to 3×10^4 spore ml^{-1} (Shamim *et al.*, 2008) using haemocytometer (Neubauer improved, Superior Marienfeld, Germany). One drop of Tween-20 per 100 ml of suspension was added as wetting agent. Spore suspension was stored in -5˚C for carrying out inoculation in the season.

3.1.2 Preparation of plant and inoculation

For raising uniform seedlings, earthen pots (diameter 15 cm) were filled up with mixture of soil, sand, compost (1:1:1) and sown with susceptible wheat variety (Agra local) and kept in growth chambers at uniform irrigation (100 ml per pot twice in a week), light intensity (5-10 Klux), temperature (22$\pm$2°C), photoperiod (12 h) and RH (60-70%). For each set of experiments (various levels of temperature exposure) sowing was done at 5-days intervals for maintaining uniformity in age of plants. Inoculation was given in thirty-day old plants batch wise. The plants were sprayed uniformly by spore suspension of the pathogen (5 ml per pot) using hand atomizer.

3.1.3 Temperature and RH-duration response on spot blotch infection

After inoculation of wheat plants, leaves were dried in front of air flow and the plants were kept in BOD (Biological Oxygen Demand) incubators set to different levels of temperature and hours of RH 95% or above (RH-duration) with a 12 h photoperiod. For establishing and maintaining high RH inside the incubator, a humidifier instrument (Donewell Rotaries, Mumbai, India) was used. Temperature levels of 16, 18, 20, 23, 26, 29, 32, 34 and 36°C were combined with 6, 10, 15, 20, 24 and 30*h* RH-duration and a Kestrel®4000 NV pocket weather tracker (Nielsen-Kellerman

Co., USA) was used for regulating temperature and RH-duration. At the end of experimental period (after exposure to the specified RH-duration), pots were removed from the RH exposure (incubator) and transferred to second incubator with same temperature but with relative humidity about 60-70%. The effect of temperature and RH-duration on the spot blotch disease development was studied by counting and calculating the number of spots cm^{-2} of leaf area. These data were converted to infection index between 0-1 dividing number of spots at each temperature by spots number at 29°C (as the most favorable temperature for infection) where 0 and 1 indicating no infection and maximum number of spots cm^{-2} of leaf area respectively. The data was analyzed in a split plot experiment (keeping temperature as the main plot and RH-duration as sub plot) with 3 replications each comprised of 5 pots with 5 seedlings in each pot. In initial results, no infection was seen on wheat plants exposed to temperature below 18°C or above 34°C and in RH-duration less than 10 h. Therefore, these levels of factors were selectively removed and statistical analysis was done only for temperature ranges between 18°-34°C under RH-duration 15, 20, 24 and 30 h. The experiments were repeated 2-4 times based on the variability of the results.

For homogeneity in data, two wet incubators were used, one for 29°C as the most favourable temperature for infection and another for other levels of temperature. In every set of experiment (temperature) the number of spots was normalized by the number of spots obtained at 29°C (Karvanen, 2003).

3.1.4 Measurement of spots number

Three leaves were randomly selected from each pot (15 leaves for each replication) and for counting spot number, leaves were scanned at 300 dpi and the image was saved in PNG format. For precise measurement of spot number image analysis software (Assess 2.0) has been used (Lakhdar, 2008). Thresholds for leaf colour were established in the HSI (Hue, Saturation and Intensity) colour space and image was segmented based on threshold values and using classic panel, leaf area was measured and the number of spots per leaf was counted and finally estimated as number of spots cm^{-2}.

3.1.5 Data analysis and model development

Analysis of variance (split-plot design) for effect of temperatures and RH-duration in relation to number of spots cm^{-2} leaf was performed. For all other analysis, number of spot cm^{-2} was normalized into infection index in 0-1 scale. To relate effect of

temperature on infection index, temperature function (Yin *et al.*, 1995; Yan and Hunt, 1999) and classical response curve for biological systems (Butler and Jadhav, 1991) were used which utilize pathogen's cardinal temperatures to estimate the shape and response.

Temperature function:

$$f(T) = [(T_{max} - T)/(T_{max} - T_{opt})] \times [(T - T_{min})/(T_{opt} - T_{min})]^{(Topt - Tmin)/(Tmax - Topt)} \quad \textbf{Eq (1)}$$

if $T_{min} \leq T \leq T_{max}$ and 0 otherwise, where f(T) = number of spot cm^{-2} normalized to 0-1, T = temperature (°C) during high RH-duration, T_{min} = minimum temperature above which symptom appeared, T_{max} = maximum temperature below which visible spots can be seen, and T_{opt} = optimum temperature for infection.

Classical response curve for biological systems with three cardinal temperatures - optimum (T_{opt}), maximum (T_{max}) and minimum (T_{min}):

$$Y = \alpha (T - T_{min}) (T_{max} - T)^{\beta} \quad \textbf{Eq (2)}$$

Where $\alpha = Y_{max} / [(T_{opt} - T_{min}) (T_{max} - T_{opt})^{\beta}]$

$$\beta = (T_{max} - T_{opt}) / (T_{opt} - T_{min})$$

An asymptotic relationship of spot blotch severity (*Y)* in 0-1 scale and RH-duration *(D)* with a minimum period of RH-duration, *m*, within which no infection occurred, was described by the following equation (Butler and Jadhav, 1991):

$$Y = Y_{max} \{ 1 - exp [-b (D - m)] \} \quad \textbf{Eq (3)}$$

Where Y_{max} = the asymptote and *b* is a measure of the rate of change of *Y* with *D*

The values of f (*T*) derived from equation (1) in different temperatures were assigned to the asymptote (Y_{max}) in equation (3), and represented the maximum possible infection with non-limiting RH-duration at the chosen temperature. The values of *m* and *b* were evaluated and assigned in equation (3) then the equation was used to obtain values of *Y* for different periods of RH (*D*) up to 30 h.

Nonlinear iterative procedures like Levenberg - Marquardt (L-M) procedure was followed (Seber and Wild, 1989; Madden *et al.*, 2007) in SPSS 16.0 to capture the feature of asymptotic behavior between *Y* and *D*. Goodness-of-fit was evaluated by the magnitude of asymptotic confidence intervals on parameter estimates and by inspection of observed values and predicted values plotted simultaneously against *T* and *D*. Heteroscedasticity was evaluated by inspecting standardized residual errors plotted against predicted values.

3.1.6 Estimation of incubation period under controlled experiment

For estimation of incubation period, raised plants in pots were inoculated at 29°C with 12 *h* RH-duration and immediately after infection, plants were exposed to temperature levels 18, 20, 23, 26, 29, 32, 34 and 36°C combining 15, 20, 24 and 30 *h* RH-duration. When the inoculated plants were transferred to second incubator, three leaves were randomly selected from each pot (15 leaves for each replication), marked by marker and after symptom appearance, number of spots was noted down. Same leaves were examined daily for counting spot number till reaching maximum number of spot. For estimation of incubation period (IP_{20}) a working definition, the time duration from infection up to 20% of spots expression was considered as one incubation period and calculated from cumulative frequency percentage. Based on field experience it was observed that cumulative frequency percentage for lower median value (IP_{20}) approximately matches with the initiation of spots on leaves or otherwise appearance of the disease. The experiments were repeated 2-4 times based on the variability of the results.

At the end of experiment, marked leaves were harvested from plants, scanned at 300 dpi and the image was saved in PNG format. Image analysis software (Assess 2.0) was used (Lakhdar, 2008) for calculation of leaf area. Thresholds for leaf color were established in the HSI (Hue, Saturation and Intensity), color space and image was segmented based on threshold values and using classic panel, leaf area was measured, the number of spots per leaf counted and finally number of spot cm^{-2} of leaf area estimated.

As IP_{20} nearly matched with the time of initial spot appearance so it was considered for simulation. IP_{20} values calculated in each temperature under laboratory experiments were plotted against temperature and linear quadratic model was fitted.

Rate for incubation period completion was expressed as the reciprocal of incubation period and estimated as incubation period per hour as weather data collected was hourly. Patterns of rate in different temperature were captured by the following equation (Pfender, 2001):

$$\textbf{Hourly rate of } IP_{20} \textbf{ completion} = (a\,T - b) \times [1 - \exp\{c\,(T - T_{max})\}]$$

Where *a*, *b*, *c* are non linear regression coefficients, *T* is the temperature and T_{max} is maximum temperature in which disease development stops. Nonlinear iterative procedures like Levenberg-Marquardt (L-M) procedure were followed (Seber and

Wild, 1989; Campbell *et al.*, 1988) in SPSS 16.0. Goodness-of-fit was evaluated by the magnitude of asymptotic confidence intervals on parameter estimates and by inspection of observed values and predicted values plotted simultaneously against *T* and IP_{20}. Heteroscedasticity was evaluated by inspecting standardized residual errors plotted against predicted values. This rate was directly considered as the fraction of one incubation period completed per hour at the ambient temperature. The fractions were summed as temperature records were received, and reaching the sum to 1 was indicating completion of one incubation period.

3.1.7 Assessment of temperature rise effect on favorable hours and rate of incubation period completion throughout Indo-Gangetic plains

For impact assessment, due to elevated temperature on spot blotch infection, throughout Indo-Gangetic plains, 25 locations representing different microclimates in six states (Punjab, Haryana, Delhi, Uttar Pradesh, Bihar and West Bengal) were considered as point data. During the crop season 2012-13, hourly weather data were collected from automatic weather stations throughout Indo-Gangetic plains available in India Meteorological Department (IMD) website. For each hour, relative humidity (RH) was calculated based on this equation (Wanielista *et al.*, 1997):

$$RH = 100\ ((112 - 0.1\ T + T_D)/(112+0.9\ T))^8$$

Where *T* is temperature and T_D is dew point temperature,

During the time of analysis it was observed that in day time about 20 to 40% higher RH and about 1-3°C lower temperature are maintained as compared to RH and temperature records at 2 m height. So, for estimation of spot blotch infection favorable hours, canopy RH and temperature assumed to be at least 20% higher and 1.5°C less respectively and automatic weather data (IMD) was corrected accordingly.

Addition of 1.5°C on ambient air temperature was considered as elevated temperature under climate change scenario. For comparison between months and locations, total favorable hours were calculated and counted at ambient and elevated air temperatures (ambient air temperature + 1.5°C) after correction of canopy level difference during December to March 2013. Similarly, for month and location wise, sum of hourly rate for incubation period completion was calculated at ambient and elevated air temperatures after correction of canopy level difference during December to March 2013. A base map was prepared with the point data using ArcGIS 10.0. Thematic maps of favorable hours and sum of rates were prepared using surface interpolation (IDW geostatistical analysis) for relative demarcation of

spot blotch infection prone areas based on temperature and RH-duration for different locations and months.

To cover a wide of range of temperature rise (0.5-3.0°C) under climate change scenario, three locations viz., Cooch Behar (West Bengal), Darbhanga (Bihar) and Gurdaspur (Punjab) have been considered for estimation of favorable period and sum of rate for incubation period completion to compare regional trend.

3.2 Field experiments

3.2.1 Field testing for infection criteria and the model

Susceptible wheat variety Agra local was sown in 24 plots (size 2×1 m) in the Institute's experimental field. Plants were inoculated by spore suspension (3×10^4 spore ml^{-1}) of the pathogen during February and March in 2012 and 2013. Twenty-four sets of inoculation were considered for the validation of combined response of temperature and RH-duration in two years. For accurate measurement of weather factors (hourly temperature and relative humidity), a calibrated Kestrel pocket weather tracker was kept inside canopy and another one at the height of 2 meters. Time of first symptom appearance was noted for every set of inoculation and hourly weather data (from infection up to first symptom expression) were uploaded from kestrel tracker to a computer using kestrel interface. Considering an hour with temperature above 17°C and relative humidity 95% or above threshold criteria favourable hours were counted assigning index 1 or 0 where 1 indicated favourable and 0 as unfavourable hour. Total number of favourable hours was counted in excel sheet till the time of first spot appearance. For field testing of the model, weather data recorded in the experimental plots were used for estimation of infection index based on the relationship developed (model) and compared with observed infection index.

3.2.2 Field validation for incubation period model and spot blotch infection criteria

Susceptible wheat variety, Agra local, was sown in 20 plots (size 2×1 m) during February-March in 2012 and 2013. In both years, first 10 plots were exposed to inoculum provided through four pots having infected plants with well sporulated spots placed inside each plot maintaining equal heights with the field plants to ensure sufficient inoculum. In another 10 plot, natural infection was allowed without keeping any infected pots. For accurate measurement of hourly temperature and RH,

a calibrated Kestrel pocket weather tracker was kept inside canopy and another one at 2 m height keeping away from direct sunlight. The time of first symptom appearance was noted for every plots and fields and hourly weather data were uploaded from kestrel tracker to a computer using kestrel interface. For the first set of experiment, hourly rate of IP_{20} completion was calculated from the time of keeping pots in plots (when daily favorable period was prevalent) up to first symptom expression using model and sum of rate accumulated to 1 for assessing the period of one incubation period completion. Time of first symptom appearance (observed incubation period) was compared with the time when accumulated rate reached to 1 (predicted IP_{20} based on the rate model). A linear regression analysis of data was produced and adjusted R^2 and relationship between observed and estimated IP_{20} evaluated.

In case of natural infection, weather data was checked daily to see whether infection favourable period (temperature 18-34°C and RH 95% or above at least 10-15 *h*) was satisfied or not. As soon as favorable conditions were satisfied, calculation of hourly rate of IP_{20} was done and summed up to the time of symptom appearance in the fields. Average value of accumulated sum of IP_{20} in different fields was considered as the criteria for indicating starting time of disease appearance.

3.2.3 Ground truth data

For ground truth observation on spot blotch severity, during the crop season 2012-13, thirteen locations across Indo-Gangetic plains (Gurdaspur, Kapurthala, Ferojpur, Karnal, Hissar, Panchkhula, Faizabad, Pilibhit, Ballia, Bhagalpur, Bhojpur, Darbhanga and Cooch Behar) were selected and disease severity was noted during the end of March following double-digit scale (*DD*, 00–99) (Saari and Prescott, 1975). The first digit (D_1) indicates vertical disease progress on the plant and the second (D_2) indicates severity measured in diseased leaf area and disease severity percentage was estimated as $\left(\frac{D_1}{9}\right) \times \left(\frac{D_2}{9}\right) \times 100$ (Sharma *et al.*, 2007).

3.3 Phytotron experiments

3.3.1 Preparation/growing of plants in Phytotron

For raising uniform seedlings, polyethylene/earthen pots (diameter 15 cm) were filled up with mixture of soil, sand, compost (1:1:1) and sown with susceptible wheat variety (Agra local) and kept in growth chambers (Environ) at uniform irrigation

(100 ml per pot twice in a week), light intensity (5-10 Klux), 12 h photoperiod and RH 60-70%.

3.3.2 Treatments of the experiment

Wheat plants were kept in separate growth chambers at the following treatments:

Trt_1: ambient CO_2 – ambient temperature (360 ppm-22°C)

Trt_2: high elevated CO_2 – ambient temperature (550 ppm-22°C)

Trt_3: ambient CO_2 – elevated temperature (360 ppm-25°C)

Trt_4: high elevated CO_2 – elevated temperature (550 ppm-25°C)

3.3.3 Inoculation procedure

Inoculation was performed on/in thirty-day old plants. The plants were sprayed uniformly by spore suspension of the pathogen (5 ml per pot) using hand atomizer. For making sufficient humidity which is required for infection, pots were covered with polyethylene bags immediately after inoculation and kept inside growth chambers till symptom development.

3.3.4 Data analysis

Analysis of variance for treatments was done using completely randomized design (CRD) with 3 replications every one comprised/consisted of 3 pots with 5 seedlings in each pot.

3.3.5 Assessment of mean number of spots cm^{-2}, mean area of spots and percentage of necrotic spots

Three inoculated leaves were randomly selected from each pot (9 leaves for each replication, 27 leaves for each treatment) and marked by marker and examined daily for counting spot numbers till reaching maximum number of spots (asymptote) after which there was no increase in spot numbers. Daily taken photographs of these leaves transmitted to computer and using image analysis software (Assess 2.0 or Image J) mean area of spots was evaluated by measuring of randomly 20 selected spots in every infected leaf. Whole area of leaves also was measured and number of spots cm^{-2} of leaf area estimated.

Thresholds for leaf colour were established in the HSI (Hue, Saturation and Intensity) colour space and image was segmented based on threshold values using classic panel for the counting of number of spots (Lakhdar, 2008).

Percentage of necrotic spots on the wheat leaves was evaluated by multiplication of spots number per cm^2 of leaf area into the average area of necrotic spots.

3.3.6 Evaluating of incubation period (IP_{50}) and latent period (LP_{50})

Counting spot numbers on the selected leaves was continued daily till reaching maximum value. The time duration between inoculation and 50% of spots appearance was considered as IP_{50} and calculated from cumulative frequency and percentage evaluation using Microsoft Excel program.

For evaluation of latent period, immediately after symptom appearance, another two infected leaves were harvested/removed from plants in each pot (6 leaves for each replication, 18 leaves for each treatment). For providing sufficient humidity which is required for sporulation, selected leaves were cut to 2-3 pieces and placed inside Petri plates on the surface of wet filter papers. Petri plates were sealed by parafilm stripes and kept inside incubator with same temperatures of treatments/experiment. Wheat leaves inside Petri dishes were examined under binocular microscope (Olympus, CH20i, BIMF, Japan) every day and number of spots which had started sporulation was noted down till completion of sporulation on almost all spots. The time duration from inoculation up to sporulation of 50% of the estimated maximum number of spots was considered as LP_{50} and assessed by calculation of cumulative frequency and percentage evaluation using Microsoft Excel program.

A model for forecasting spot blotch disease in wheat

4.1. Abstract

Spot blotch of wheat, caused by *Bipolaris sorokiniana*, is considered as one of the most important diseases in warmer areas of the world, particularly in South Asia. Number of spot cm^{-2} leaf area as an attribute of disease severity was observed to be influenced markedly by various combinations of temperature and duration of high relative humidity (95% or above). Effects of temperature and RH-duration on spot blotch infection were explained by fitting the response equations in terms of infection index. Temperature response was typically unimodal with cardinal temperatures, i.e. minimum, optimum and maximum, had been appropriated at 18, 29 and 34^{o}C, respectively. Response of RH-duration was non-linear, which was characterized by a monotonic increase in infection index with the increase of RH-duration. Requirement of RH-duration for spot density had decreased with increase in temperature up to 29°C while it had increased afterwards till 34°C. After inoculation, a minimum RH-duration (*m*) was required after which spots could be seen. The fitted equations were used to produce a series of response curves and surface response in relation to temperature (*T*) and RH-duration (*D*) which could explain variation in infection index and thus proposed as a model: $Y = \left(\frac{36-T}{7}\right) \times \left(\frac{T-16}{13}\right)^{2.6} \times (1 - 0.8114^{D-m})$ where m is dependent on *T*. Through field testing observed and predicted surface response for infection index in the inoculated plots was almost similar and the linear regression of infection index produced an adjusted R^2 of 0.963. The proposed model could be used to develop weather-based disease forecaster for scheduling protective fungicide applications.

4.2. Key words: Wheat, Spot blotch, Forecasting model, *Bipolaris sorokiniana*

4.3. Introduction

Wheat is the second most important food crop of India contributing nearly one third of the total food grain production. Several biotic and abiotic factors are the stumbling blocks in realizing the potential yield of wheat. Among the biotic factors, the most widely prevalent, notorious and shifty enemy on wheat is spot blotch disease caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker syn. *Drechslera sorokiniana* (Sacc.)

Subrm and Jain (syn. *Helminthosporium sativum* Pamm., King & Bakke), teleomorph *Cochliobolus sativus* (Ito & Kuribayashi) Drechsl. ex Dastur (Dubin and van Ginkel, 1991; Joshi *et al.,* 2004; Sharma and Duveiller, 2006; Pandey *et al.,* 2005). Different types of symptoms are produced by the pathogen on different plant parts, which include seedling blight, rotting of root, spot blotch on leaf, and black point in grain. Infected or infested seeds, crop residues, collateral hosts, and free dormant conidia in the soil have been reported to be the natural source of inoculum of the pathogen (Reis, 1991). *B. sorokiniana* has a wide host range; besides wheat, infection on oat, barley, maize, rice, wild canary grass and linseed plant has also been reported (Prem *et al.,* 2009). Spot blotch is widely prevalent in warm and humid environments. An estimated 25 m ha of wheat growing areas, covering Africa, South America, Australia, Canada and Asia particularly Indian sub-continent, is affected by the disease (Duveiller *et al.,* 2005; Van Ginkel and Rajaram, 1998). About 10 m ha of wheat growing belt in Indian subcontinent is affected by the disease, out of which 9 m ha belonging to Indo-Gangetic plains predominantly with rice-wheat cropping system shared the maximum (Nagarajan and Kumar, 1998; Ruckstuhl, 1998; Singh *et al.,* 1998; Shamim *et al.,* 2010). The spot blotch imparts yield loss in the tune of 20-80% and the extent of loss depends upon the severity of occurrence (Saari, 1998; Duveiller and Sharma, 2009). To combat with such losses, currently, efforts are being made to develop resistant varieties by deploying resistant sources known against spot blotch (Kumar *et al.,* 2007; Lillemo *et al.,* 2013).

Temperature and relative humidity are the major weather parameters those influence spot blotch infection (Mehta, 1998; Singh *et al.,* 1997; Singh *et al.,* 1998). A continuous rain for 5-6 days followed by warmer temperatures (day average of 20-30^{o}C) has been reported to be conducive weather for rapid development of spot blotch epidemic (Mehta, 1998). Infection was quicker and severe at 28°C than at 20°C (Nema and Joshi, 1973). For maximum development of blight has been reported to be at temperature around 28°C and relative humidity 92% (Singh *et al.,* 1997) and no infection was successful at the average temperature below 18°C (Singh *et al.,* 1998). High humidity for 72 h was adequate to establish infection in adult plants and light infection was noted when temperature was above 30°C (Senthil, 2004). Reis (1991) suggested that for foliar blight outbreak, wheat leaves must remain wet for >18 h at a mean temperature of 18˚C or higher. Therefore, it is well established fact that the disease is highly influenced by weather factors as leaf

wetness or high relative humidity and temperature are necessary for infection by *B. sorokiniana*. However, most of the report are based only on empirical considerations as the combined effects of temperature and duration of relative humidity or leaf wetness on spot blotch infection has never been quantified.

Since the disease is highly influenced by weather, there is an immediate need to develop accurate relationship with weather factors for precise evaluation of environmental interaction on segregating population, generated in the resistant breeding programmes. Prediction of infection using forecasting models is essential tool to assess the temporal progress of the disease in segregating population as well as to estimate the climate change impact on the disease. A perusal of literature indicated a quantum of work was done on epidemiology of the disease, however, earlier studies are focused entirely on empirical considerations ignoring the interaction-pattern between weather parameters specially levels of temperatures and duration of high relative humidity. Such information is a prerequisite to develop a forecasting model for the disease which in turn is very relevant for developing an advisory system for the stakeholder. In the present study we are reporting the development of a model for forecasting spot blotch disease based on critical weather parameters.

4.4. Materials and methods

All investigations were conducted at the laboratory, net house and experimental field of Plant Pathology Division, Indian Agricultural Research Institute (28°38'23"N, 77°09'27"E, 228.61 m above mean sea-level), New Delhi, India.

4.4.1. Preparation of inoculum

B. sorokiniana was isolated from one heavily infected field of Institute's experimental farm and its pathogenicity was proved and then the culture was compared for similarity with a pathogenic isolate of Indian Type Culture Collection. For sporulation, the fungus was grown 10 days in water agar at 24°C. The spore suspension was prepared and adjusted to 3×10^4 spores ml^{-1} of water using hemocytometer (Shamim *et al.,* 2008). One drop of Tween-20 per 100 ml of suspension was added as wetting agent. Spore suspension was stored in -5°C for carrying out inoculation in the season.

4.4.2. Preparation of plant and inoculation

For raising uniform seedlings, earthen pots (diameter 15 cm) were filled up with mixture of soil, sand, compost (1:1:1) and sown with susceptible wheat variety (Agra local) and kept in growth chambers (Environ) at uniform irrigation (100 ml per pot twice in a week), light intensity (5-10 Klux), temperature (22±2°C), with 12 h photoperiod and RH 60-70%. For each set of experiments (various levels of temperature exposure) sowing was done at 4-days intervals for keeping uniformity in age of plants. Inoculation was given in thirty-day old plants batch wise. The plants were sprayed uniformly by spore suspension of the pathogen (5 ml per pot) using hand atomizer.

4.4.3. Temperature and RH-duration response on spot blotch infection

After inoculation of the plants, leaves were dried in front of air flow and the inoculated plants were kept in BOD (Biological Oxygen Demand) incubator set to different temperature and RH-duration levels with a 12-h photoperiod. For establishing and maintaining high RH inside the incubator, a humidifier instrument (Donewell Rotaries, Mumbai, India) was used. Temperature levels of 16, 18, 20, 23, 26, 29, 32, 34 and 36°C were combined with 6, 10, 15, 20, 24, 30 h of high relative humidity 95% or above (RH-duration). Temperature and RH-duration were measured using Kestrel®4000 NV pocket weather tracker (Nielsen-Kellerman Co., USA) up to 30 h. At the end of experimental period (after exposure to the specified temperature and RH-duration), pots were removed from the RH exposure (incubator) and transferred to another incubator with same temperature but with humidity about 60-70%. The effect of temperature and RH-duration on spot blotch disease development was studied in a split plot experiment (treating temperature as the main plot and RH-duration as the sub plot treatment) with 3 replications. In initial results, no infection was seen on wheat plants exposed to temperature below 18°C or above 34°C and in RH-duration less than 10 h. Therefore, these levels of factors were selectively removed and statistical analysis was done only for temperature ranges between 18°-34°C under RH-duration 15, 20, 24 and 30 h.

For homogeneity in data, two wet incubators were used, one for 29°C as the most favourable temperature for infection and another for other levels of temperature. In every set of experiment (temperature) the number of spots was normalized by the number of spots obtained at 29°C (Karvanen, 2003).

4.4.4. Measurement of spots number

Three leaves were randomly selected from each pot (15 leaves for each replication) and for counting spot number, leaves were scanned at 300 dpi and the image was saved in PNG format. For precise measurement of spot number image analysis software (Assess 2.0) has been used (Lakhdar, 2008). Thresholds for leaf colour were established in the HSI (Hue, Saturation and Intensity) colour space and image was segmented based on threshold values and using classic panel, leaf area was measured and the number of spots per leaf was counted and finally estimated as number of spot cm^{-2}.

4.4.5. Data analysis and model development

Analysis of variance (split-plot design) for effect of temperatures and RH-duration in relation to number of spot cm^{-2} leaf was performed. For all other analysis, number of spot cm^{-2} was normalized into infection index in 0-1 scale. To relate effect of temperature on infection index, temperature function (Yin *et al.*, 1995; Yan and Hunt, 1999) and classical response curve for biological systems (Butler and Jadhav, 1991) were used which utilize pathogen's cardinal temperatures to estimate the shape and response.

Temperature function:

$$f(T) = [(T_{max} - T)/(T_{max} - T_{opt})] \times [(T - T_{min})/(T_{opt} - T_{min})]^{(Topt - Tmin)/(Tmax - Topt)} \quad \textbf{Eq (1)}$$

if $T_{min} \leq T \leq T_{max}$ and 0 otherwise, where f(T) = number of spot cm^{-2} normalized to 0-1, T = temperature (°C) during high RH-duration, T_{min} = minimum temperature above which symptom appeared, T_{max} = maximum temperature below which visible spots can be seen, and T_{opt} = optimum temperature for infection.

Classical response curve for biological systems with three cardinal temperatures - optimum (T_{opt}), maximum (T_{max}) and minimum (T_{min}):

$$Y = \alpha\,(T - T_{min})\,(T_{max} - T)^{\beta} \quad \textbf{Eq (2)}$$

Where $\alpha = Y_{max} / [(T_{opt} - T_{min})\,(T_{max} - T_{opt})^{\beta}]$

$$\beta = (T_{max} - T_{opt}) / (T_{opt} - T_{min})$$

An asymptotic relationship of spot blotch severity (*Y)* in 0-1 scale and RH-duration *(D)* with a minimum period of RH-duration, *m*, within which no infection occurred, was described by the following equation (Butler and Jadhav, 1991):

$$Y = Y_{max}\{1 - exp[-b(D - m)]\} \quad \textbf{(3)}$$

Where Y_{max} = the asymptote and *b* is a measure of the rate of change of *Y* with *D*

The values of f (*T*) derived from equation (1) in different temperatures were

assigned to the asymptote (Y_{max}) in Eq (3), and represented the maximum possible infection with non-limiting RH-duration at the chosen temperature. The values of *m* and *b* were evaluated and assigned in Eq (3) then the equation was used to obtain values of *Y* for different periods of RH (*D*) up to 30 h.

Nonlinear iterative procedures like Levenberg - Marquardt (L-M) procedure was followed (Seber and Wild, 1989; Madden *et al.,* 2007) in SPSS 16.0 to capture the feature of asymptotic behavior between *Y* and *D*. Goodness-of-fit was evaluated by the magnitude of asymptotic confidence intervals on parameter estimates and by inspection of observed values and predicted values plotted simultaneously against *T* and *D*. Heteroscedasticity was evaluated by inspecting standardized residual errors plotted against predicted values.

4.4.6. Field testing for infection criteria and the model

Susceptible wheat variety Agra local was sown in 24 plots (size 2×1 m) in the Institute's experimental field. Plants were inoculated by spore suspension (3×10^4 spore ml^{-1}) of the pathogen during February and March in 2012 and 2013. Twenty-four sets of inoculation were considered for the validation of combined response of temperature and RH-duration in two years. For accurate measurement of weather factors (hourly temperature and relative humidity), a calibrated Kestrel pocket weather tracker was kept inside canopy and another one at the height of 2 meters. Time of first symptom appearance was noted for every set of inoculation and hourly weather data (from infection up to first symptom expression) were uploaded from kestrel tracker to a computer using kestrel interface. Considering an hour with temperature above 17°C and relative humidity 95% or above threshold criteria favourable hours were counted assigning index 1 or 0 where 1 indicated favourable and 0 as unfavourable hour. Total number of favourable hours was counted in excel sheet till the time of first spot appearance. For field testing of the model, weather data recorded in the experimental plots were used for estimation of infection index based on the relationship developed (model) and compared with observed infection index.

4.5. Results

4.5.1. Temperature response on spot blotch infection

Inoculated wheat plants exposed in different levels of temperature had shown marked effect on the development of spots on leaves (Table 4.1). Spot blotch symptoms did not develop either 16°C or 36°C, even when exposed to different periods of RH undertaken in the study. Typical spot blotch symptoms appeared only in the temperature range 18° to 34°C. In temperature 18° or 34°C minimum number of spots was observed only with 20-30 h exposure to RH. However, number of spots increased significantly (Duncan test, $p<0.05$) from temperature 20° to 29°C when RH exposure was 15-30 h. Maximum number of spots was noted at 29°C when exposed to 24 h RH, however, above this temperature there was significant decrease in spot number. At 29°C spot development was observed even as minimum as 10 h of RH exposure. A unimodal relationship, found between spot blotch index (Y) and temperature (at 24 h of RH), could be used to estimate the thresholds temperatures (minimum, optimum and maximum) on pathogen's growth on the host (Fig.4.1 and 4.2). In relation to infection index, the temperature function (equation 1) was found to be better fit than that of classical biological response function (equation 2). Based on the model, cardinal temperatures i.e., maximum (T_{max}), minimum (T_{min}) and optimum temperature (T_{opt}) for infection was approximated to be 36°, 18° and 29°C, respectively.

4.5.2. RH-duration response on spot blotch infection

Inoculated plants exposed to different duration of RH along with different temperatures had shown a sigmoid response (Fig. 4.3). Spot blotch infection did not occur at RH exposure with 6 and 10 h as no visible spot was noted. However, as mentioned earlier, spot development was observed at 10 h RH exposure when temperature was set at 29°C. Above 10 h of exposure, spot blotch development increased significantly (Duncan test, $p<0.05$) with the increase in duration of RH and it had reached saturation at 24 h. There was significant variation in spot number for temperature-RH-duration interaction as observed in the experiment (Table 4.1). With 10, 15, 20, 24 and 30 h of RH exposures significantly higher number of spots was observed at 29°C as compared to other temperatures considered in the experiment. An asymptotic relationship, found between spot blotch index (Y) and RH-duration (D), was explained by nonlinear model (Eq 3) with parameter estimates for Y_{max}, b

and *m* given in Table 2 for each temperature. After inoculation, there was a minimum period of RH-duration (*m*) after which visible spots could be seen. Temperature had significant influence on $Y_{\max}$ and *m* as these values increased due to increase in temperature. Results of the nonlinear regression analysis were consistent with the inference drawn from analysis of variance. The results indicated that values of *b* increased with increase in temperature between 18° and 26°C and decreases afterward (Table 4.2). The value of *m* was assumed to decrease linearly with the increasing temperature up to 29°C while above that; it increases linearly (Fig.4.4).

4.5.3. Combined response curves as model for both temperature and RH-duration for forecasting spot blotch infection

Effect of temperature and RH-duration on a 3-D plot from controlled experiment had indicated the pattern of weather response on spot blotch infection index (Fig.4.5). The simulated response surface (Fig.4.6), generated based on the average values of *b* and *m* values given in Table 4.2, had reflected similar pattern of infection index realized under controlled experiment (Fig.4.5). However, for mathematical simulation of the pattern for spot blotch infection, temperature response (Eq 1) and RH-duration response (Eq 3) was combined to generate response curves (Fig.4.7). Therefore, simulated response curves could be used as model for assessing the effect of weather on spot blotch infection in wheat. Hence, Eq (3) after simplification could be presented as model to explain infection index in relation to temperature (*T*), RH-duration (*D*) and minimum RH-duration after which symptoms were visible (*m*) where *m* is dependent on temperature. The simplified version of equation (3) after replacement of Y_{max} as function of temperature proposed:

$$Y = \left(\frac{36-T}{7}\right) \times \left(\frac{T-16}{13}\right)^{2.6} \times \left(1 - 0.8114^{(D-m)}\right) \qquad \text{Eq (4)}$$

There would be little change in the index if the RH-duration were extended beyond 24 h, as may happen with continuously wet weather for more than 1 day. Based on these curves, daily value of infection index may be predicted from the duration of RH and the average temperature prevailed on a particular day. Estimated daily infection index is the likelihood of spot blotch infection and further progress of infection could be forecast provided availability of accurate weather forecast data.

Table 4.1: Temperature response on spot blotch severity (mean number of spots cm^{-2} leaf area) on wheat leaves in relation to various levels of high relative humidity (≥ 95%) exposures inoculated with *B. sorokiniana*.

Temperature (°C)	Number of spots cm^{-2} of leaf area					
	Duration of high relative humidity (h)					
	6	10	15	20	24	30
16	0	0	0	0	0	0
18	0	0	0 a	1.2 b (±0.2)	1.3 bc (±0.2)	2 cd (±0.1)
20	0	0	1.1 ab (±0.1)	3.3 d (±0.2)	4.2 d (±0.2)	4.2 d (±0.2)
23	0	0	4 e (±0.2)	5.2 ef (±0.2)	6.8 f (±0.2)	6.8 f (±0.2)
26	0	0	7.5 g (±0.2)	9 h (±0.3)	11 hi (±0.2)	11 hi (±0.2)
29	0	1	9.2 j (±0.5)	12 jk (±0.4)	13 k (±0.6)	13 k (±0.6)
32	0	0	3.3 ab (±0.1)	8.1 hi (±0.3)	10 i (±0.3)	10 i (±0.3)
34	0	0	0 a	2 ab (±0.1)	3.8 b (±0.2)	3.8 b (±0.2)
36	0	0	0	0	0	0

Note: The values with same letter are not statistically different at 5% level of significance (Duncan test) and values inside bracket are standard error.

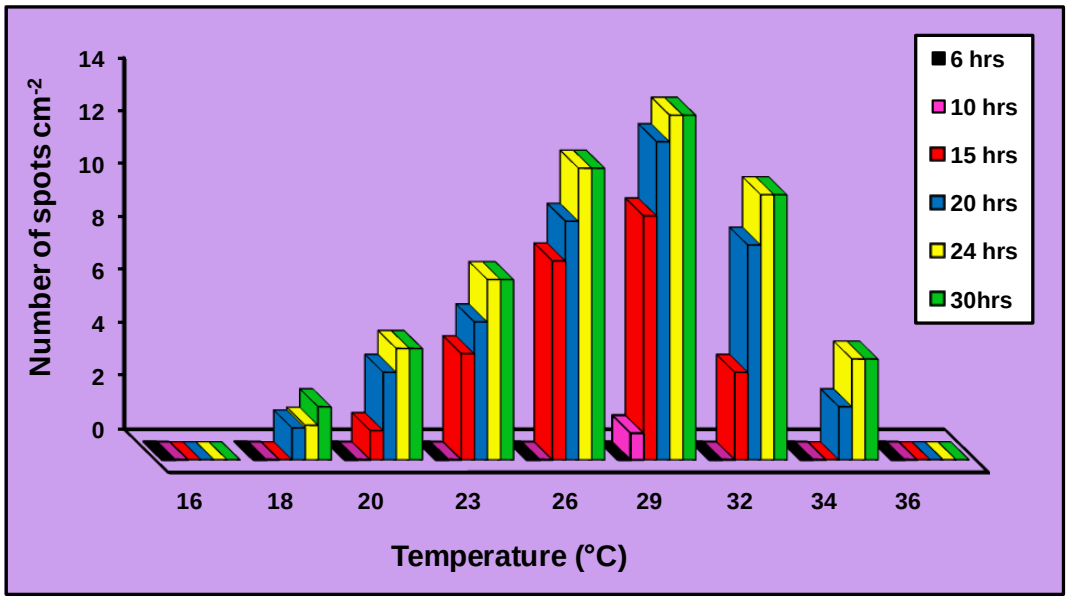


Fig. 4.1: Effect of temperature on spot blotch severity (number of spots cm^{-2}) on wheat leaves at different levels of high relative humidity (≥ 95%) duration.

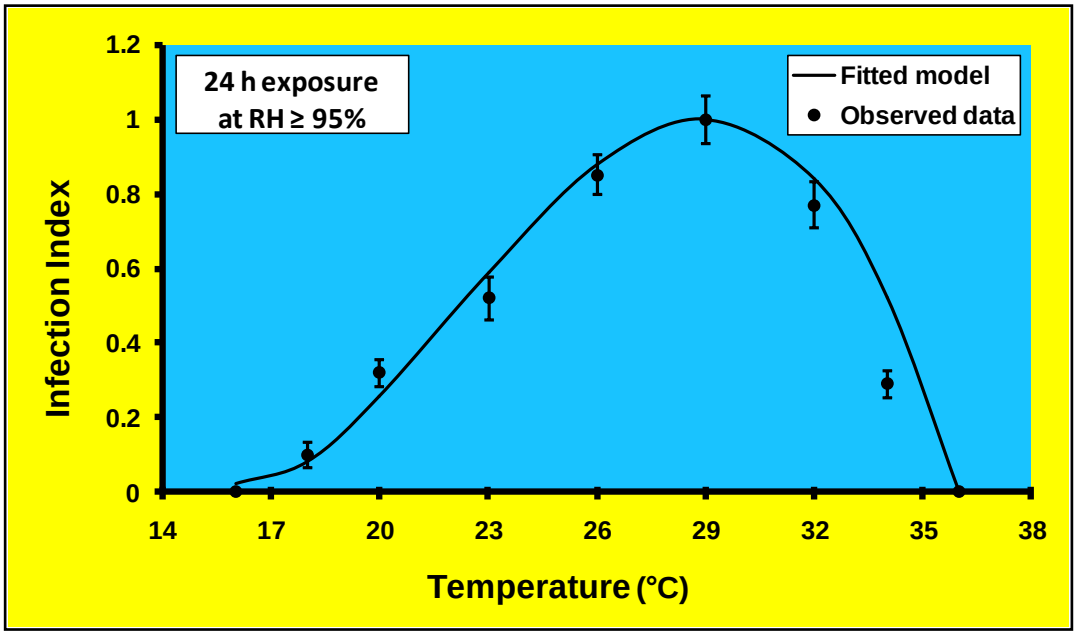


Fig. 4.2: Temperature response on spot blotch severity (number of spots cm^{-2} on wheat leaves in terms of infection index) based on temperature function model (equation 1) at 24 h of high relative humidity (≥ 95%).

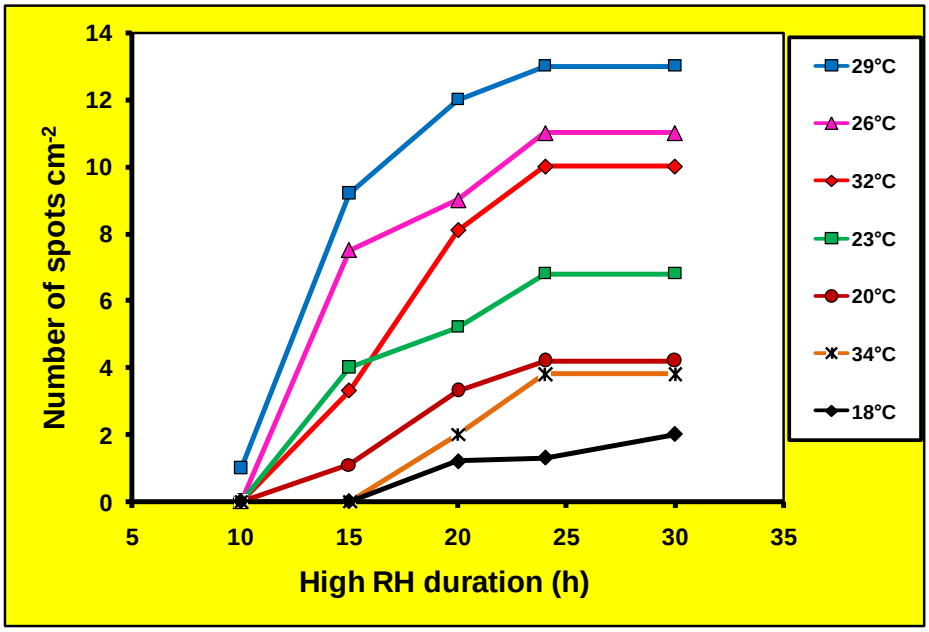


Fig. 4.3: High relative humidity (≥95%) duration (RH-duration) response on spot blotch severity (number of spots cm^{-2} on wheat leaves in terms of infection index) at different levels of temperatures.

Table 4.2: Parameters estimate with standard errors (S.E.), obtained by fitting Eq (3) to data relating spots number cm^{-2} of leaf area (in terms of infection index 0-1 scale) in wheat in relation to high relative humidity duration at different temperatures.

Temperature (°C)	Y_{max} Observed	Y_{max} estimate	S.E.	*b*	S.E.	*m*	S.E.	R^2
18	0.154	0.179	0.050	0.116	0.064	15	1.000	0.962
20	0.323	0.356	0.032	0.173	0.043	13	0.577	0.974
23	0.523	0.521	0.042	0.256	0.075	12	0.577	0.864
26	0.846	0.822	0.050	0.364	0.100	12	0.577	0.810
29	1.000	1.034	0.020	0.197	0.014	9	0.288	0.985
32	0.769	0.820	0.041	0.204	0.031	13	0.577	0.987
34	0.292	0.344	0.077	0.150	0.077	15	1.000	0.959

* Y_{max} = asymptote; *b*= rate of change in spots density with increasing duration of high relative humidity; *m*= minimum duration (h) of high relative humidity after which visible spots could be seen.

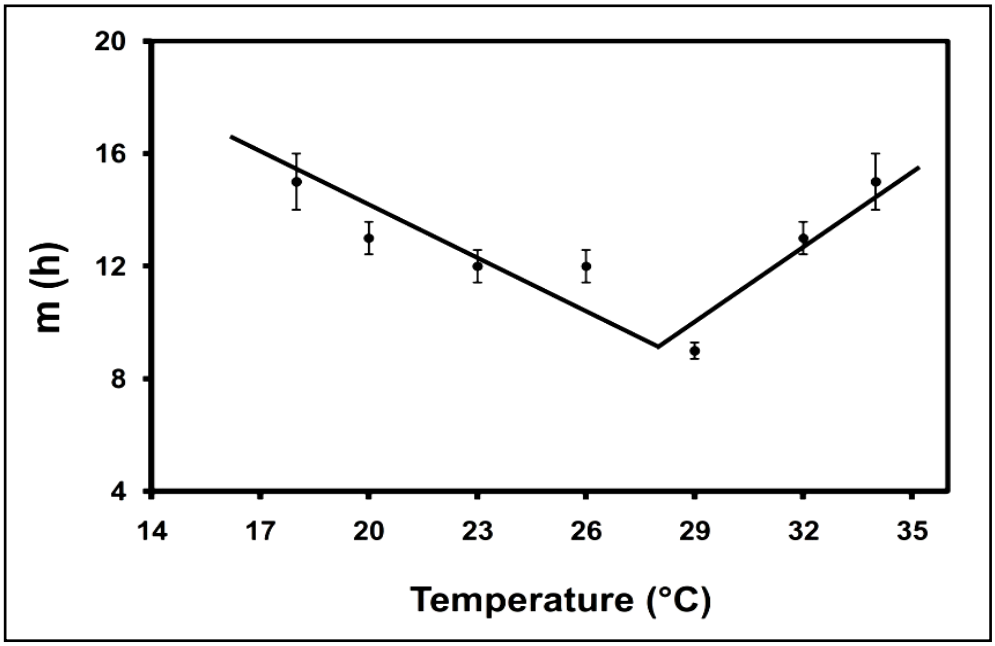


Fig. 4.4: Effect of temperature on the minimum time (*m*) of high relative humidity (≥ 95%) after which spots could be seen (bars indicate standard errors).

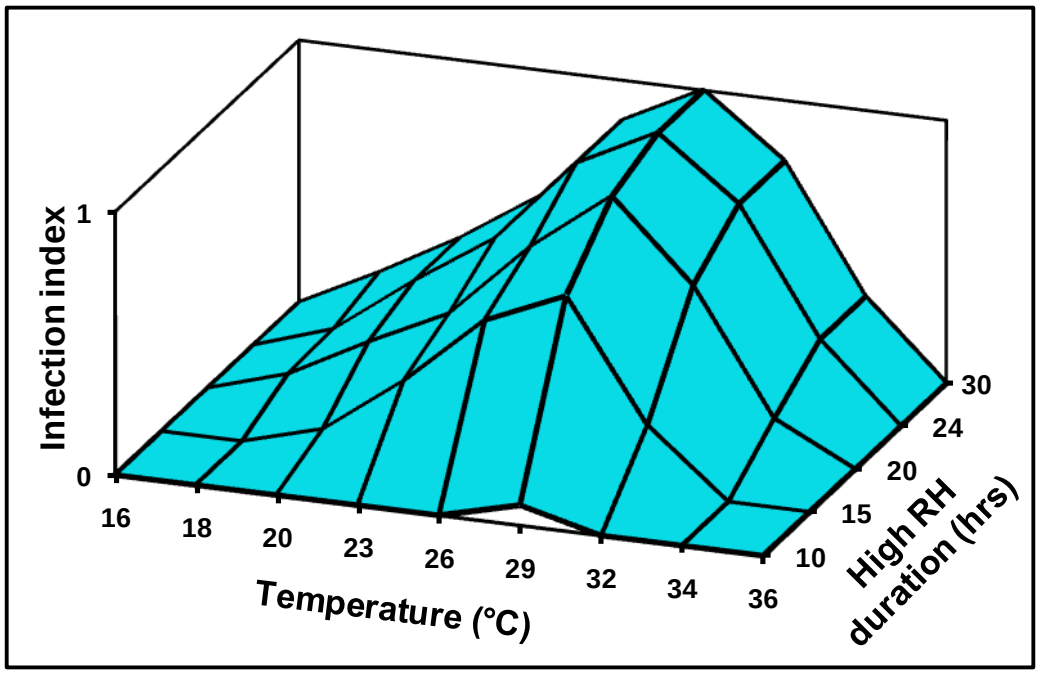


Fig. 4.5: Response surface indicating relationship among temperature, high relative humidity (≥ 95%) duration (h) and spot blotch infection index in wheat based on data from controlled experiment.

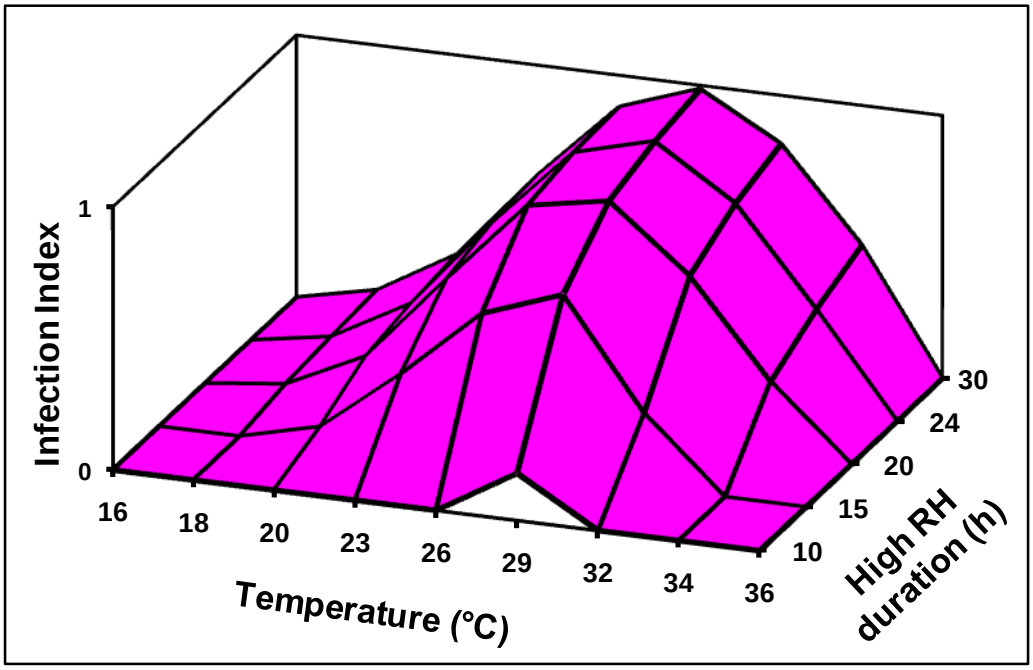


Fig. 4.6: Response surface indicating relationship among temperature, high relative humidity (≥95%) duration (h) and spot blotch infection index in wheat based on data mathematically simulated by combining Eq (4).

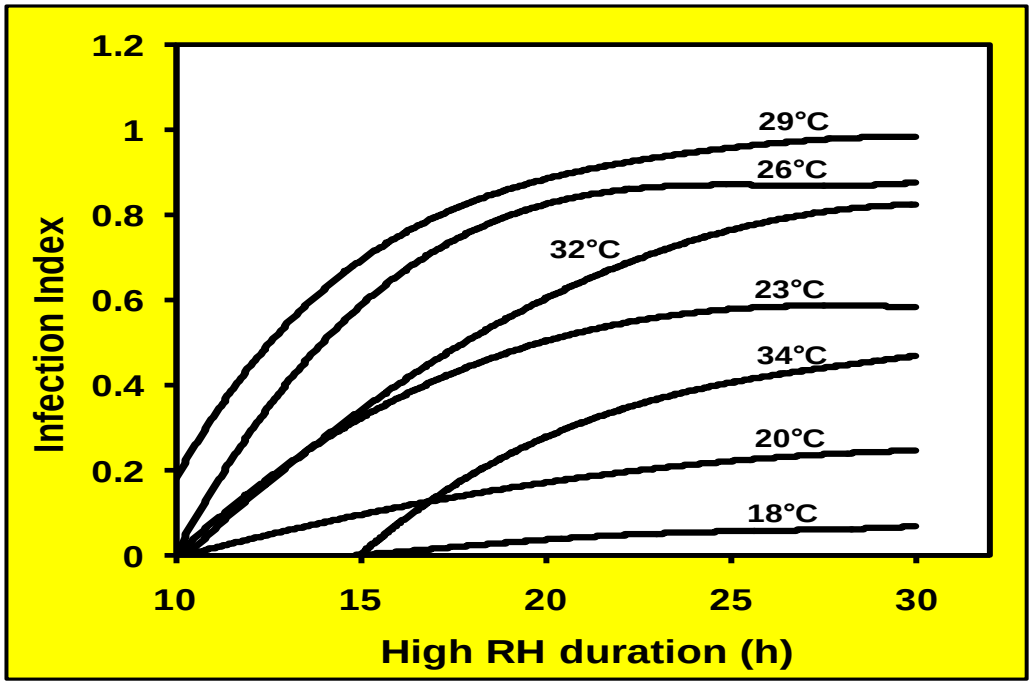


Fig. 4.7: Simulated curves showing relative amounts of spot blotch infection (infection index) for various temperatures with different periods of high relative humidity (≥95%) based on Eq (4)

4.5.4. Field testing for infection criteria and the model

Inoculation of wheat plants under field condition was made during the congenial periods for spot blotch infection. Non-occurrence of natural infection other than artificial inoculation was confirmed as only the inoculated plants within the plot have shown infection after incubation period 3-7 days. Visible and countable spots were appeared in 20 out of 24 cases of inoculation as there were sufficient favorable hours for the next four days. During this four days period, temperature and RH-duration were in the range of 17°-30.8°C and 17-26 h (Fig.4.8 and Table 4.3). Based on the infection development criteria determined and the relationship worked out (model), at the prevailing temperature during RH-duration, at least 14-15 h was required to get infection. The available RH-durations in these 20 cases were between 17-26 h which was sufficient for infection. Since the favourable period within 48 h was about 17-26 h therefore, spot blotch infection criteria was satisfied and symptoms were noted. On the other hand, in four cases, after inoculation, at least 13-14 favourable hours was required based on the criteria model. However, during the period favourable hours was only for 7-12 h within 48 h of inoculation including interruption (Fig.4.9 and Table 4.3). Therefore, non appearance of symptom in four cases could be explained as the period after inoculation was not sufficiently conducive for spot blotch development. Observed and predicted surface response for infection index in the inoculated plots was almost similar (Fig.4.10) and the linear regression of infection index (Fig.4.11) produced an adjusted R^2 of 0.963 with the following relationship:

Observed infection index = 0.96 (Predicted infection index) – 0.015.

Therefore, observed and predicted spot blotch infection had good correspondence.

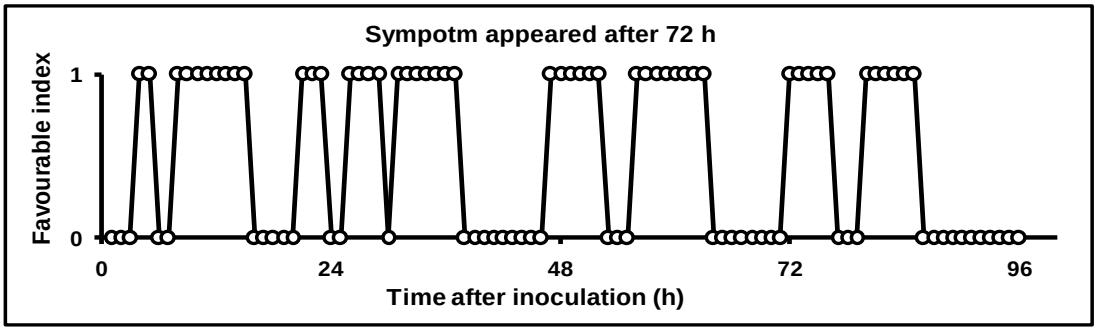


Fig. 4.8: Field testing for spot blotch infection in wheat (March 2013). Favourable period (temperature > 17°C and relative humidity ≥ 95%) inside wheat canopy inoculated with *B. sorokiniana* in first 48 h of inoculation = 26 and at 96 h of inoculation = 49. The mean temperature in whole period of experiment was 21.8°C in which at least 14-15 h favourable period required for infection.

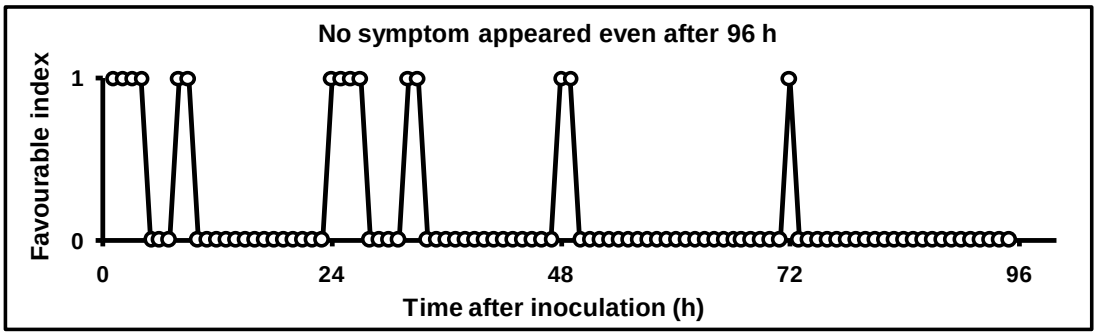


Fig.4.9: Field testing for spot blotch infection in wheat (February 2012). Favourable period (temperature >17°C and relative humidity ≥ 95%) inside wheat canopy inoculated with *B. sorokiniana* in first 48 h of inoculation = 13 and at 48-96 h of inoculation = 2. The mean temperature in whole period of experiment was 22.9°C in which at least 13-14 h favourable period required for infection.

Table 4.3: Field testing of spot blotch infection criteria in wheat in relation to various levels of favorable hours (temperature >17°C and relative humidity ≥95%) during February-March 2012-13.

Number of inoculation	Number of favorable hours		Interruption hours	Temperature range (°C)	Remarks
	within 48 h	between 48-96 hours			
20	17-26	16-25	1-10	17- 30.8	Disease
4	7-12	2-5	14-24	16.5- 30.2	No disease

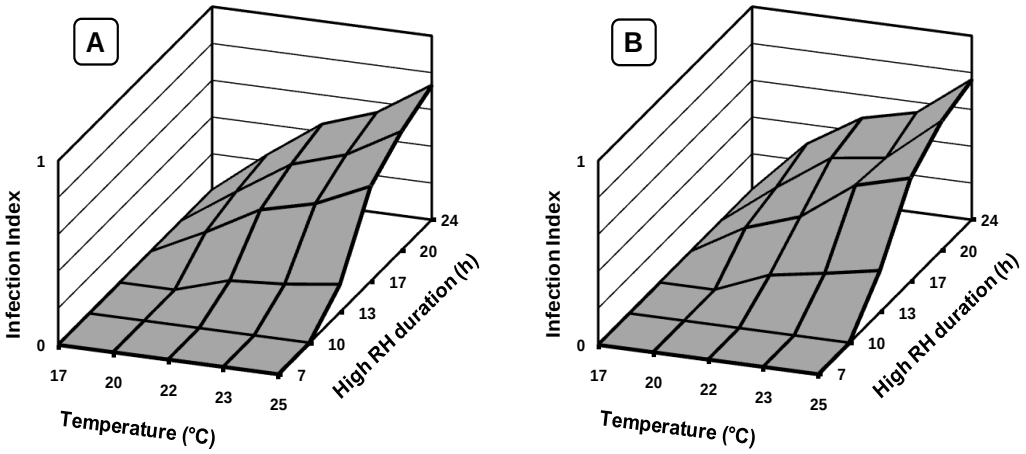


Fig. 4.10: Predicted (A) and observed (B) surface response for infection index based on field conditions (prevalent temperature and high relative humidity duration) in wheat inoculated with *B. sorokiniana*.

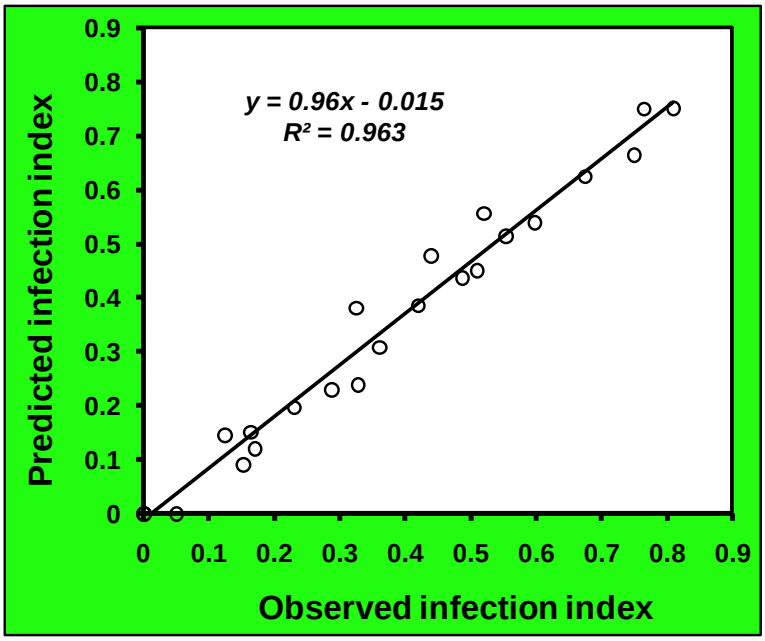


Fig. 4.11: Observed infection index for spot blotch development on wheat plants inoculated with *B. sorokiniana* in the field and predicted infection index derived from model based on field weather data. Each data point represents an independent trial using observation on 24 plots during February to March 2012-2013.

4.6. Discussion

A model for forecasting spot blotch infection in wheat, based on temperature and high RH-duration, has been developed for the first time. Infection model for fungal diseases is one of the most critical components for disease forecasting as infection process is usually limited by duration of RH or surface wetness in most terrestrial environments (Magarey *et al.,* 2005; Madden *et al.,* 2007). An infection process on wet leaves proceeds more quickly at higher temperatures so that temperature during the wetting period as well as the duration of the wetting period must be considered (Wilks and Shen, 1991) and different combinations of wetting duration and temperature are important for plant diseases (Huber and Gillespie, 1992). Therefore, very often the two factors are combined to construct an index for the occurrence of plant diseases (Gillespie and Sutton, 1979; Wilks and Shen, 1991; Magarey *et al.,* 2005; Guyader *et al.,* 2013).

The predictive model has been developed on the basis of two important assumptions; firstly, there was presence of sufficient virulent inoculum in the field and secondly, the conducive weather factors had prevailed to establish the spot blotch infection. Upon these assumptions, we have tried to characterize the most critical weather factors, which triggered spot blotch infection and studied their interaction (response surface) thoroughly for establishing a predictive model. Similar modeling approach to describe the shape of the response surface was used for other tropical fungal pathosystem diseases too (Magarey *et al.,* 2005; Guyader *et al.,* 2013). It was possible to draw conclusions about the biological relevance of the shape of the response surface even though parameter estimates were derived from the controlled environment data.

Temperature is one of the most important component for spot blotch development. Earlier report indicated that rapid and severe spot blotch infection could occur at 28°C (Singh *et al.*, 1998; Senthil, 2004). However, a broad range of temperature (18° to 32°C) has also been reported for foliar blight outbreaks in wheat from Brazil (Reis, 1991). All these reports did not consider the role of cardinal temperatures in spot blotch development and thus could not be considered to explain the infection process under varying field condition. We have developed a unimodal temperature curve for spot blotch disease, which was found to be useful for fixing

cardinal temperatures, and hence could be a valuable tool for explaining infections under field conditions.

Duration of high relative humidity, in addition to temperature, is the second important factor for spot blotch infection. Infection process on wet leaves proceeds more quickly at higher temperatures so that temperature during the wetting period as well as the duration of the wetting period must be considered (Wilks and Shen, 1991). Periods of leaf wetting can be estimated using specialized instruments or, more roughly, from the duration of relative humidity more than 90% (Sutton *et al.,* 1984). In fact, high relative humidity duration above a threshold limit has been found to be useful to explain infection process for many tropical fungal pathosystem in the field (Wilks and Shen, 1991; Gleason *et al.,* 1994). Available information in the literature indicated either leaf wetness more than 18 h with temperature 18° to 32°C (Reis, 1991) or continuous rain for 5-6 days followed by higher temperature (20°-30°C) triggers rapid development of spot blotch (Mehta, 1998). However, none of them considers entire dimension of moisture requirement necessary for infection, hence, a RH response curve explaining the infection process could not be developed based on such observation. In present findings, RH response behavior on spot density has been worked in details to explain all variations and representative response curves, which is necessary for prediction of the disease, has been established. Based on the response curves, daily value of infection index may be predicted from the duration of RH and the average temperature prevailed on a particular day especially during the high RH period. Daily infection index may be used as the likelihood of spot blotch infection and its progress could be forecast provided availability of accurate weather forecast data.

The third important factor for spot blotch development is the interaction of temperature and RH-duration. None of the literature has yet focused on this critical aspect of infection process. Hence, our study has generated important and valuable information in this regard for the first time. Maximum spot blotch infection index, asymptote (Y_{max}) and minimum time lag (m) is influenced by temperature. It has been realized from the relationship curve between m and temperature (Figure 3), that RH-duration requirement for spot blotch infection had decreased with increase in temperature up to 29°C while it had increased afterwards till 34°C. Since higher RH-duration was required for spot blotch development at temperature below or above

29°C, therefore, the fitted line was realistic and thus the relationship worked out is reliable to explain spot blotch infection.

Validation of the model under field condition indicated that canopy weather data is more reliable than the available weather data (recorded at 2 m height) for prediction of accurate spot blotch infection. However, acquisition of canopy level weather data is practically not feasible through normal weather data recording devices. Hence, data on canopy weather conditions especially for temperature and RH-duration should be obtained after adjustment of differences between weather data available at 2 m height and at canopy level.

In summary, a model was developed to understand the critical parameters for spot blotch infection in wheat. Outputs from this model developed in the study could be used in future either as a framework for developing another simpler, rules-based forecaster or as a base model in developing a management advisory programme for the stakeholder.

Incubation period model for estimation of spot blotch developmental rate in wheat

5.1. Abstract

Spot blotch (*Bipolaris sorokiniana*) in wheat is one of the most important diseases in warmer areas of the world particularly in South Asia. Effect of different levels of temperature, duration of high relative humidity (≥ 95%) and incubation period (IP_{20}-time from start of infection to 20% spot appearance) were estimated for prediction of spot blotch infection under elevated temperature. Temperature 18-34°C and a minimum 15 *h* of high RH was found to be required for infection. IP_{20} was decreased from 7 days at 18°C to 2 days at 29°C and afterward increased with the increase in temperature. Hourly rate of IP_{20} completion was best described as a linear increase in rate with increase of temperature up to approximately 29°C, then an exponential decline up to the maximum temperature of approximately 36°C in which disease development stopped. A model for hourly IP_{20} completion rate established as *(0.002 Temp –0.03) {1 – exp [0.151(Temp – 36)]}* could reasonably explain the rate of spot development for diurnal temperature fluctuations tested under natural infection in the field.

5.2. Key words: Wheat, Spot blotch, Incubation period, Rate of incubation period completion

5.3. Introduction

Bipolaris sorokiniana (Sacc.) Shoemaker syn. *Drechslera sorokiniana* (Sacc.) Subrm and Jain (syn. *Helminthosporium sativum* Pamm., King & Bakke), teleomorph *Cochliobolus sativus* (Ito & Kuribayashi) Drechsl. ex Dastur is the causal agent of common root rot, leaf spot, seedling blight, head blight of wheat and barley and black point of grains. The disease is distributed worldwide and reported from South and East Asia (Saari, 1998), North and Latin America, Africa (Duczek and Jones-Flors, 1993), India (Joshi *et al.*, 2002), China (Chang and Wn, 1998) and Brazil (Mehta, 1998). The pathogen perpetuates both externally as conidia and internally as mycelium in the seeds, as well as in infected crop residues, volunteer plants, secondary hosts and free dormant conidia in the soil (Reis, 1991). However, the role

of infected seed as a primary source of inoculums appears to be important and according to Shaner (1981) as it is the main source of inoculums of leaf blight pathogens. About 25 million hectare of cultivated wheat area is affected worldwide by spot blotch (Van Ginkel and Rajaram, 1998). It has become a major production constraint in South Asia's intensive rice-wheat cropping systems and around 12 million hectare wheat area is affected (Nagarajan and Kumar, 1998; Ruckstuhl, 1998; Singh *et al.,* 1998) by spot blotch every year and yield losses in the tune of 20% to 80% were reported (Duveiller and Sharma, 2009) in this region. Few studies indicated that spot blotch infection in wheat is highly influenced by weather factors mainly temperature and high relative humidity (Singh *et al.,* 1997; Singh *et al.,* 1998; Mehta, 1998; Reis, 1991; Senthil, 2004). Disease response in the field can vary differently because they experience a larger number and a wider range of environmental cues (including variations in light quality, temperature and RH level). However, little is known about the importance of small changes in temperature under field conditions, or the biometeorological basis of responses to such changes.

The disease is increasingly becoming a cause of concern particularly in the warm and humid environments of Indian sub-continent (Duveiller *et al.,* 2005) where the mean temperature of the coolest month is higher than 17.5˚C (Dubin *et al.,* 1998).

Change in favorable conditions in relation to rise in temperature may be useful to assess the impact of climate change on the disease. Temperature induced incubation and /or latent period models could describe developmental or generation rate of the pathogen on host (Madden *et al.,* 2007) as incubation period is generally characterized by a decrease in duration as temperature rises from the minimum to the optimum, then an increasing duration with higher temperatures (Analytis, 1977; Logan *et al.,* 1976; Pfender, 2001). Effect of changing environment on the pathogen characteristics such as frequency of generations and predictions on how changes in temperature will affect plant health requires knowledge on already observed effects of climate change on plant diseases, extrapolation from expert knowledge and experimental studies, and computer models (Garrett *et al.,* 2006; Savary *et al.,* 2011). Latent period completion rate for stem rust in tollgrass and fescue was found to increase gradually from T_{min} up to T_{opt} and then decline more rapidly to reach zero at the T_{max} or lethal temperature (Pfender, 2001). Therefore, effect of diurnal temperature fluctuation either below or above these threshold levels ($T_{min,}$ T_{max} and T_{opt}) could be related with incubation or latent period to reflect the development of

the pathogen on host. Such temperature-based developmental rate model is also likely to facilitate temperature rise effect on diseases that may happen under climate change regime. A perusal of literature has indicated that there was no systematic study to establish any such models for forecasting spot blotch in wheat and simulate the impact of climate change on the disease in the region.

In the present communication, objective was to determine favorable conditions for spot blotch infection in wheat and develop a model for incubation period.

5.4. Materials and methods

All investigations were conducted at the laboratory, net house and experimental field of Plant Pathology Division, Indian Agricultural Research Institute (28°38'23"N, 77°09'27"E, 228.61 m above mean sea-level), New Delhi, India.

5.4.1. Preparation of inoculum

B. sorokiniana was isolated from one heavily infected field of Institute's experimental farm and its pathogenicity was proved comparing similarity with a pathogenic isolate of Indian Type Culture Collection. For sporulation, the fungus was grown 10 days in water agar at 24°C. The spore suspension was prepared and adjusted to 3×10^4 spore ml^{-1} (Shamim *et al.*, 2008) using haemocytometer (Neubauer improved, Superior Marienfeld, Germany). One drop of Tween-20 per 100 ml of suspension was added as wetting agent. Spore suspension was stored in -5˚C for carrying out inoculation in the season.

5.4.2. Preparation of plants and inoculation

For raising uniform seedlings, earthen pots (diameter 15 cm) were filled up with mixture of soil, sand, compost (1:1:1) and sown with susceptible wheat variety (Agra local) and kept in growth chambers (Connviron, E15, Canada) at uniform irrigation (100 ml per pot twice in a week), light intensity (5-10 Klux), temperature (22$\pm$2°C), photoperiod (12 h) and RH (60-70%). For each set of experiments (various levels of temperature exposure) sowing was done at 5-days intervals for maintaining uniformity in age of plants. Inoculation was given in thirty-day old plants batch wise. The plants were sprayed uniformly by spore suspension of the pathogen (5 ml per pot) using hand atomizer.

5.4.3. Estimation of incubation period under controlled experiment

For estimation of incubation period, raised plants in pots were kept in BOD (Biological Oxygen Demand) incubators, inoculated at 29°C with 12 *h* RH-duration

and immediately after infection, plants were exposed to temperature levels 18, 20, 23, 26, 29, 32 and 34°C combining 15, 20, 24 and 30 *h* RH-duration. A Kestrel®4000 NV pocket weather tracker (Nielsen-Kellerman Co., USA) was used for regulating temperature and RH-duration. For establishing and maintaining high RH inside the incubator, a humidifier instrument (Donewell Rotaries, Mumbai, India) was used. At the end of experimental period (after exposure to the specified RH-duration), pots were removed from the RH exposure (incubator) and transferred to second incubator with same temperature but with relative humidity about 60-70%.

Three leaves were randomly selected from each pot (15 leaves for each replication), marked by marker and after symptom appearance, number of spots was noted down. Same leaves were examined daily for counting spot number till reaching maximum number of spot.

For estimation of incubation period (IP_{20}) a working definition, the time duration from infection up to 20% of spots expression was considered as one incubation period and calculated from cumulative frequency percentage of number of spots cm^{-2} (Fig. 5.1). Based on field experience it was observed that cumulative frequency percentage for lower median value (IP_{20}) approximately matches with the initiation of spots on leaves or otherwise appearance of the disease. The experiments were repeated 2-4 times based on the variability of the results.

At the end of experiment, marked leaves were harvested from plants, scanned at 300 dpi and the image was saved in PNG format. Image analysis software (Assess 2.0) was used (Lakhdar, 2008) for calculation of leaf area. Thresholds for leaf color were established in the HSI (Hue, Saturation and Intensity), color space and image was segmented based on threshold values and using classic panel, leaf area was measured, the number of spots per leaf counted and finally number of spot cm^{-2} of leaf area estimated.

As IP_{20} nearly matched with the time of initial spot appearance so it was considered for simulation. IP_{20} values calculated in each temperature under laboratory experiments were plotted against temperature and linear quadratic model was fitted.

Rate for incubation period completion was expressed as the reciprocal of incubation period and estimated as incubation period per hour as weather data collected was hourly. Patterns of rate in different temperature were captured by the following equation (Pfender, 2001):

$$\textit{Hourly rate of } IP_{20} \textit{ completion} = (a\,T - b) \times [1 - exp\,\{\,c\,(\,T - T_{max})\}]$$

Where *a*, *b*, *c* are non linear regression coefficients, *T* is the temperature and T_{max} is maximum temperature in which disease development stops. Nonlinear iterative procedures like Levenberg-Marquardt (L-M) procedure were followed (Seber and Wild, 1989; Jeger, 1986; Campbell *et al.*, 1988) in SPSS 16.0. Goodness-of-fit was evaluated by the magnitude of asymptotic confidence intervals on parameter estimates and by inspection of observed values and predicted values plotted simultaneously against *T* and IP_{20}. Heteroscedasticity was evaluated by inspecting standardized residual errors plotted against predicted values. This rate was directly considered as the fraction of one incubation period completed per hour at the ambient temperature. The fractions were summed as temperature records were received, and reaching the sum to 1 was indicating completion of one incubation period.

5.4.4. Field validation for incubation period model

Susceptible wheat variety, Agra local, was sown in 20 plots (size 2×1 m) during February-March in 2012 and 2013. In both years, first 10 plots were exposed to inoculum provided through four pots having infected plants with well sporulated spots placed inside each plot maintaining equal heights with the field plants to ensure sufficient inoculum. In another 10 plot, natural infection was allowed without keeping any infected pots. For accurate measurement of hourly temperature and RH, a calibrated Kestrel pocket weather tracker was kept inside canopy and another one at 2 m height keeping away from direct sunlight. The time of first symptom appearance was noted for every plots and fields and hourly weather data were uploaded from kestrel tracker to a computer using kestrel interface. For the first set of experiment, hourly rate of IP_{20} completion was calculated from the time of keeping pots in plots (when daily favorable period was prevalent) up to first symptom expression using model and sum of rate accumulated to 1 for assessing the period of one incubation period completion. Time of first symptom appearance (observed incubation period) was compared with the time when accumulated rate reached to 1 (predicted IP_{20} based on the rate model). A linear regression analysis of data was produced and adjusted R^2 and relationship between observed and estimated IP_{20} evaluated.

In case of natural infection, weather data was checked daily to see whether infection favourable period (temperature 18-34°C and RH 95% or above at least 10-15 *h*) was satisfied or not. As soon as favorable conditions were satisfied, calculation

of hourly rate of IP_{20} was done and summed up to the time of symptom appearance in the fields. Average value of accumulated sum of IP_{20} in different fields was considered as the criteria for indicating starting time of disease appearance.

5.5. Results

5.5.1. Estimation of incubation period under controlled experiment

Incubation period for 20 percent of the spot appearance (IP_{20}) was studied in 15, 20, 24 and 30 *h* RH-duration. Based on goodness-of-fit evaluated by the magnitude of asymptotic confidence intervals on regression parameter estimates and by inspection of observed values and predicted values plotted indicated that difference in incubation period was not affected within 15-30 *h* RH-duration. Minimum IP_{20} value was about 2 days in 29°C and maximum about 7 days in 18°C with 15-30 *h* RH-duration (Fig. 5.2). IP_{20} was found to decrease as temperature increased from 18 to 29°C and then increased with increase in temperature. Fitting quadratic equation could best explain the relationship of temperature (T) and incubation period (IP_{20}) in four levels of RH-duration considered (Fig. 5.3). Regression equations in different levels of RH-duration were:

$$IP_{20} = 0.062T^2 - 3.440T + 49.70 \quad \text{with } R^2 = 0.963 \quad (15\ h)$$

$$IP_{20} = 0.051T^2 - 2.859T + 41.72 \quad \text{with } R^2 = 0.980 \quad (20\ h)$$

$$IP_{20} = 0.047T^2 - 2.613T + 38.43 \quad \text{with } R^2 = 0.984 \quad (24\ h)$$

$$IP_{20} = 0.041T^2 - 2.369T + 37.00 \quad \text{with } R^2 = 0.960 \quad (30\ h)$$

Rate of incubation period (IP_{20}) or the reciprocals of incubation period increased almost linearly with temperature from 16 to 29°C and then exponentially declined from 29 to 36°C (Fig. 5.4). By fitting Pfender's model the rate equation was derived as follows:

$$\text{Hourly rate of } IP_{20} \text{ completion} = (0.002T - 0.03)\ \{1 - \exp[0.151(T - 36)]\}$$

Where T is the average temperature during the one hour period.

The values predicted incubation period were with good agreement observed incubation period values (R^2 =0.962). Although the model has shown fairly accurate estimation towards lower and higher temperature but there was little under-estimation around optimum of temperature for infection.

5.4.2. Field validation for incubation period model and infection criteria

In both the year of validation, during 2012 and 2013, the longest incubation period observed in the plots was about 5 days and the shortest was 3 days. Temperature-

response equations fitted to IP_{20} data gave good correspondence between modeled incubation period times and observed incubation periods (Fig. 5.5). A linear regression analysis of these data (data not shown) produced an adjusted R^2 of 0.982 and the following relationship:

Observed incubation period = 0.968 (modeled incubation period) – 0.041

However, during February 2013, number of daily favorable hours was below the minimum favorable duration requirement (10-15*h*) for infection (Fig. 5.6). Number of favorable hours increased from March 1-6 with the increase in seasonal temperature. Visible and countable spots appeared in 12-16 days and 11-15 days during 2012 and 2013 respectively (Table 5.1). Sum of hourly IP_{20} rate was calculated after the occurrence of minimum favorable hour requirement and appearance of spots has been found to match with a rate accumulation of 3.08-3.15. Therefore, under Delhi conditions, approximately accumulation of about 3.00 may be counted for the time of spot blotch appearance.

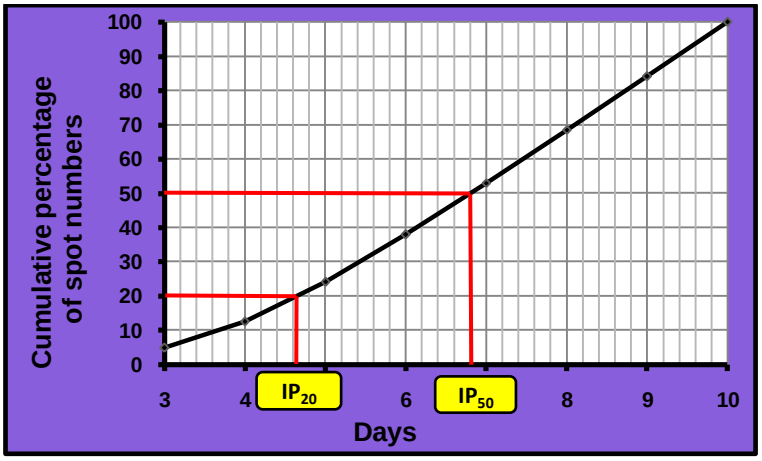


Fig. 5.1: A guide model for calculation of IP_{20} and IP_{50} from cumulative percentage of spot numbers

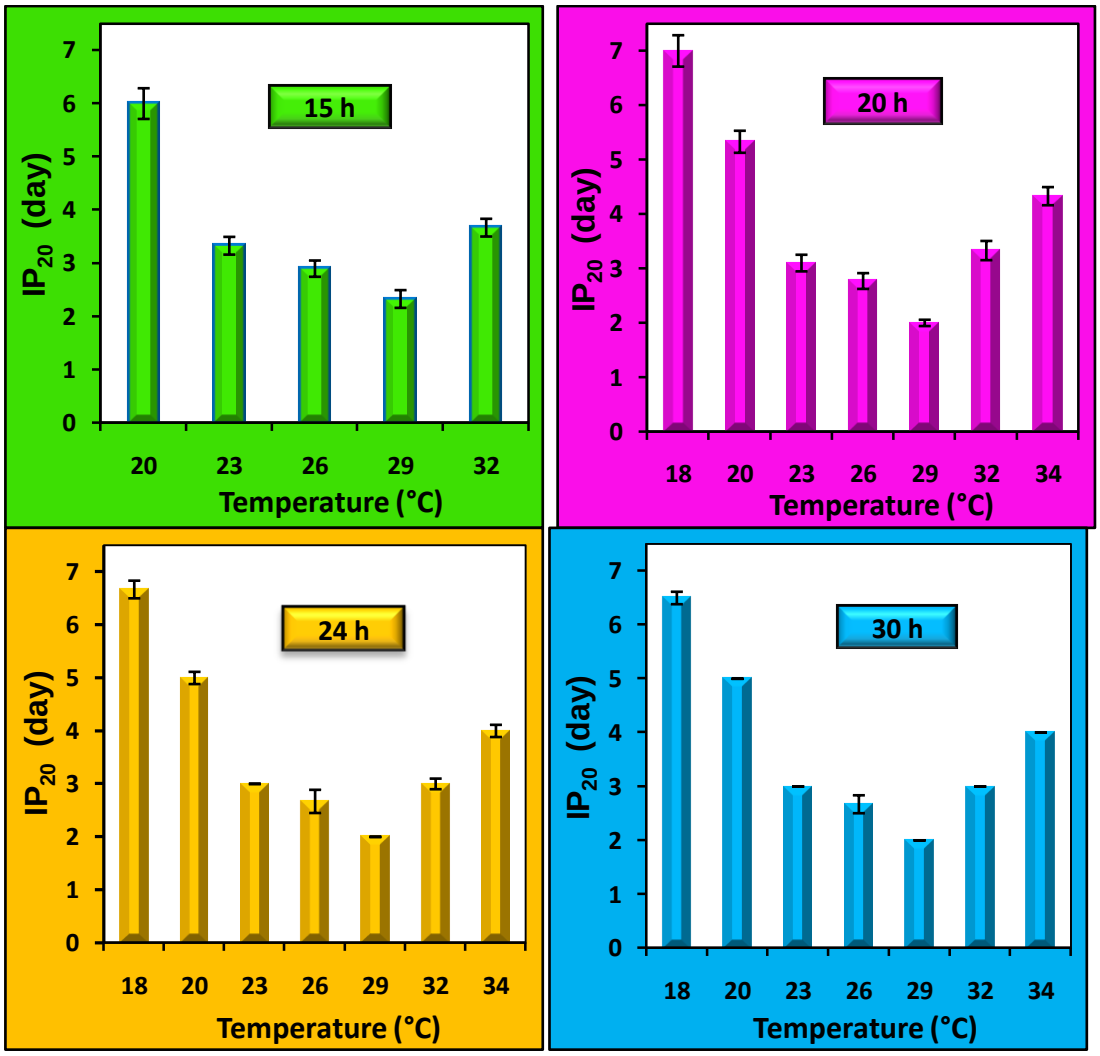


Fig. 5.2: Incubation period (IP_{20}) for spot blotch development in wheat leaves in relation to temperature under high relative humidity duration 15, 20, 24 and 30 *h.* (Bars show standard error).

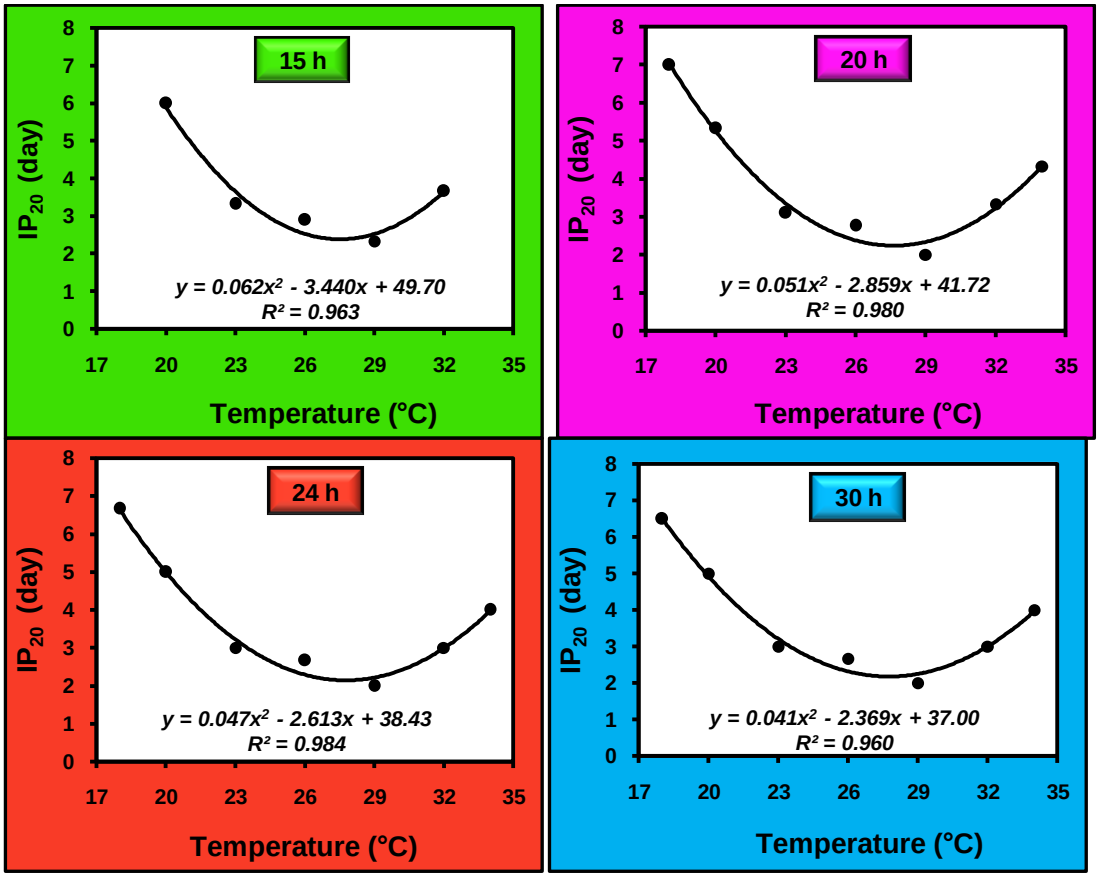


Fig.5.3: Regression lines and relationship between temperature and incubation period (IP_{20}) in spot blotch disease of wheat under high relative humidity duration 15, 20, 24 and 30 *h*.

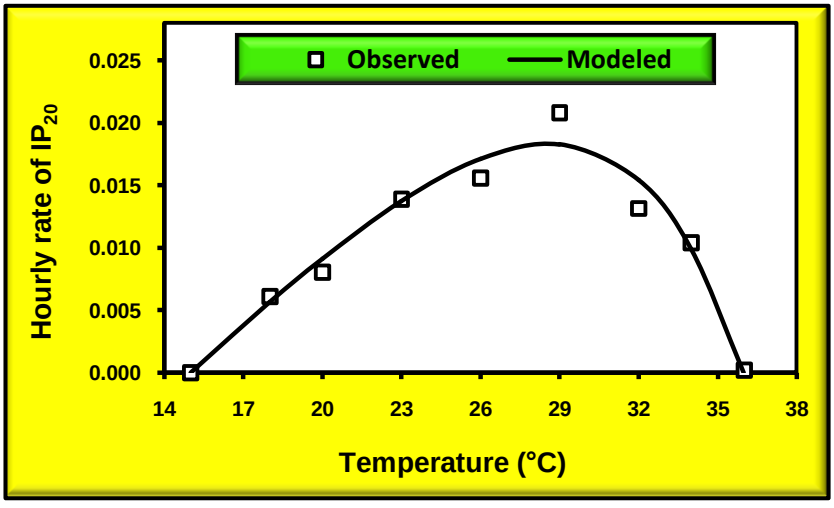


Fig. 5.4: Hourly rate of IP_{20} completion (fraction of incubation period per hour) for spot blotch development in wheat plants in relation to temperature based on observed (calculated from a constant-temperature trials) and predicted data.

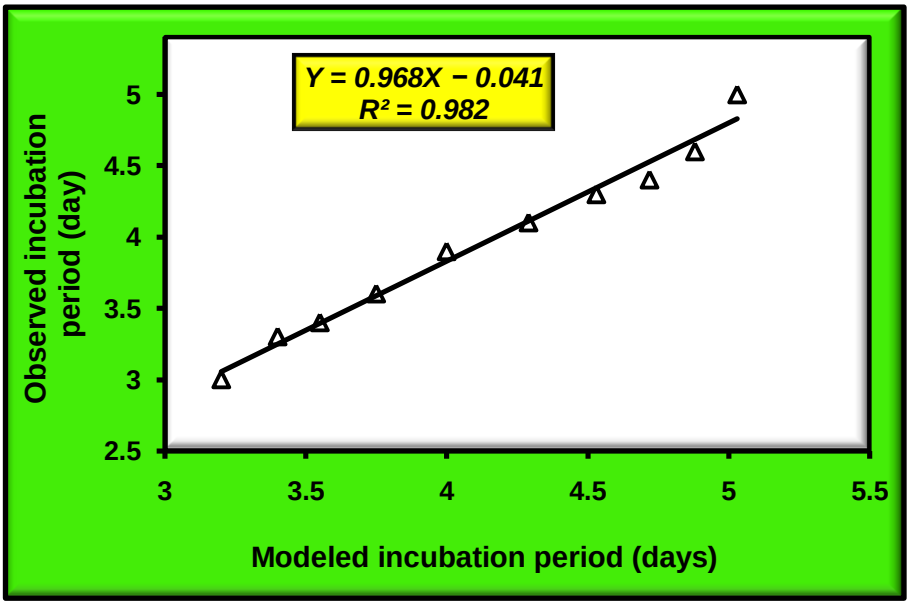


Fig. 5.5: Observed incubation periods (IP_{20}) for spot blotch development on wheat plants inoculated with *Bipolaris sorokiniana* (keeping four infected pots inside plots) and predicted incubation-periods from hourly ambient canopy temperatures under field conditions, during February to March 2013.

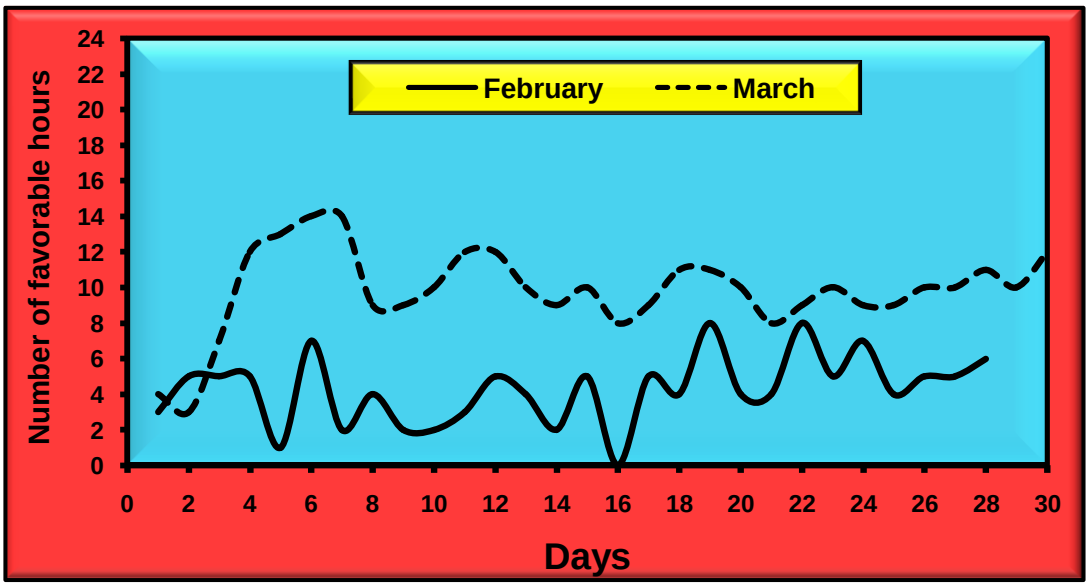


Fig. 5.6: Number of favorable hours (temperature 18-34°C and relative humidity ≥ 95%) inside canopy of wheat field naturally infected with spot blotch disease during February and March 2013 at New Delhi location.

Table 5.1: Spot blotch disease appearance in naturally infected wheat fields in New Delhi location during March 2012 and 2013 and forecasting based on hourly rate of incubation period (IP_{20}) completion and accumulation of rates from starting of favorable period (temperature 18-34°C, relative humidity $\geq$ 95% at least 10-15 hours daily) up to the time of symptom appearance.

Field No.	Date of starting favorable period in the field		Date of symptoms appearance		Days required for disease expression		Sum of hourly rate for IP_{20}	
	2012	2013	2012	2013	2012	2013	2012	2013
1	Mar 4	Mar 1	Mar19	Mar 15	16	14	3.47	3.32
2	Mar 4	Mar 1	Mar 17	Mar 16	14	15	3.26	3.24
3	Mar 4	Mar 1	Mar 18	Mar 14	15	12	3.36	3.01
4	Mar 4	Mar 1	Mar 17	Mar 16	14	13	3.23	3.15
5	Mar 5	Mar 3	Mar 16	Mar 15	12	12	2.93	2.86
6	Mar 5	Mar 3	Mar 17	Mar 15	13	12	2.95	3.05
7	Mar 5	Mar 4	Mar 18	Mar 15	14	11	3.16	2.98
8	Mar 5	Mar 4	Mar 19	Mar 16	15	12	3.18	2.94
9	Mar 6	Mar 4	Mar 18	Mar 17	13	13	3.08	3.19
10	Mar 6	Mar 5	Mar 17	Mar 17	12	12	2.84	3.09
Mean					13.8	12.6	3.15	3.08

5.4. Discussion

Current finding establishes the fact that occurrence of infection favorable temperature and RH-duration with a specified range in crop canopy facilitates spot blotch infection. Once infection is occurred subsequent progress of the disease is largely dependent on temperature. It has been evident that for spot blotch infection temperature rise both due to seasonal rise and climate change plays important role as duration of infection favorable conditions as well as rate of incubation period completion otherwise symptom expression is significantly influenced by change in temperature.

Temperature has greatly influenced spot blotch development as the number of spots increased with increase in temperature. Direct effect of increasing temperature on multiplication and sporulation rate of the pathogen is very well known *in vitro* and *in vivo* (Duveiller *et al.*, 1998) and higher temperature favored spot blotch severity (de Lespinay, 2004). In the present study, incubation period model for

development of 20% spots from the start of infection has been considered and rate of incubation period completion has been established in relation to temperature. The valid use of rate summation methods to predict development under fluctuant temperatures assumes that the order in which different temperatures and RH-duration occur does not affect development (Hau *et al.,* 1985). The violation of this assumption may not lead to large errors in predicted developmental rate because, under field conditions, the fungus is unlikely to be subjected to such high temperatures or scanty RH-duration for a prolonged period in its early developmental stages. Thus, the model has revealed overall effect of temperature on spot blotch density after the initial infection took place. Quadratic model for incubation period could describe the characteristics decrease in duration in spot blotch development as temperature rises from the minimum to the optimum and increase in duration with higher temperatures. Linear quadratic models have been used to describe latent period behavior in barley rust (*Puccinia hordei*) (Teng *et al.,* 1980), alfalfa rust (Webb and Nutter, 1997) and *Colletotrichum* spp infecting strawberry fruit (King *et al.,* 1997) as they could explain the characteristics pattern of increase or decrease in developmental rate due to change in temperature. Pattern of incubation period for spot blotch development is not affected by RH-duration for infection. It is evident that RH-duration is essential only for establishment of infection on leaf surface and for development of symptoms temperature plays important role. Model for rate of incubation period completion or otherwise developmental rate in field testing has shown to capture the effect of hourly temperature in completion of a fraction of incubation period. The fraction or rate of incubation period completion is relevant to estimate the effect of diurnal temperature fluctuations on spot blotch development as the weights of temperature fluctuations around T_{min}, T_{opt} and T_{max} are taken into account. Hourly estimate of incubation-period progress can be estimated through the model as it is easy to fit and to apply to temperature data in a datalogger output. Other models (Analytis, 1977; Logan *et al.,* 1976) proposed for developmental rate are not straightforward as they require transformation of data (Pfender, 2001). Thus the model could allow estimating the effect of temperature rise due to climate change.

Impact of elevated CO_2 and temperature on spot blotch disease in wheat under controlled conditions

6.1. Abstract

In wheat, spot blotch caused by *Bipolaris sorokiniana*, once unknown or localized importance has become a serious problem in wheat under current scenario. Effect of elevated CO_2 concentration and temperature on spot blotch was studied controlled conditions using phytotron chambers. Wheat plants were grown in phytotron cahmbers with different levels of CO_2 (360 and 550 ppm) and temperatures (22 and 25°C) and artificially inoculated with spore suspension of *Bipolaris sorokiniana* indicated significant changes in epidemic parameters, *i.e.*, number of spots cm^{-2} leaf area, mean spot area (mm^2), percentage of necrotic area, incubation period and latent period and percentage of necrotic area. Influence of elevated CO_2 was noted in terms of increased leaf area and spot size and decrease in spot number. Increasing in CO_2 level did not appear to affect incubation and latent period as the temperature rise had shown to influence incubation and latent period. By increasing both CO_2 and temperature significant decrease in incubation and latent period was noted. However temperature effect was prominently realized in terms of increased spot number and necrotic area and decreased incubation and latent period as well as increased disease severity. Increase in temperature alone caused increased number of spots cm^{-2} and decreased in sporulation of pathogen but did not affect mean size of necrotic spots. Elevated CO_2 as well as elevated temperature increased percentage of leaf infection and maximum infection was observed in combined treatment of elevated CO_2 and temperature. Therefore, direct effect of temperature rise on pathogen and probably indirect effect of changes due to CO_2 in host likely to contribute definitive increase in spot blotch in wheat.

6.2. Key words: Wheat, Spot blotch, elevated CO_2, elevated temperature

6.3. Introduction

Spot blotch, caused by *Cochliobolus sativus* (Ito& Kurib.) Drechsler ex Dastur [anamorph, *Bipolaris sorokiniana* (Sacc.) Shoem.] is a serious constraint to wheat (*Triticum aestivum* L.) production in the warmer plains of South Asia. The importance of spot blotch also known as foliar blight or *Helminthosporium* leaf blight in South Asia was highlighted early in reports from India (Nema and Joshi, 1973), Bangladesh (Badaruddin *et al.,* 1994) and Nepal (Devkota, 1994). It is widely prevalent and becomes a cause of concern throughout the world specially in Africa, South America, Australia, Canada and Asia particularly in Indian sub-continent having warm and humid environments (Van Ginkel and Rajaram, 1998). The disease frequently occurs in North Eastern states of India but now it has also made appearance in severe form in Punjab, Haryana and western Uttar Pradesh due to rapid expansion of rice wheat rotation (Singh and Srivastava, 1997). Eeconomic yield losses due to spot blotch was estimated 3-20 % in India,71% in Bangladesh, 20-30 % (but sometimes above 75%) in China, 16.2-29% in Nepal and 40% in Philippines (Senthil, 2004).

Drechslera sorokiniana is transmitted through contaminated or infected seed. It also survives on crop residues, straw and in soil (Reis, 1991).

Stagnant or lower wheat yields in recent years in the plains of South Asia is considered partly due to climatic factors including heat stress characterized by an increasing trend in average temperature during winter months (Nagarajan, 2005; Sharma *et al.,* 2007). Another factor causing decline in wheat productivity in rice-wheat system, the most dominant cropping system in the region, is related to soil fertility and fertilizer use (Nagarajan, 2005; Tirol-Padre *et al.,* 2006). Nagarajan (2005) also listed socio-economic and policy related factors being responsible for recent decline in wheat production in the Indo-Gangetic plain. A steadily increase of mean March temperature was observed at five sites in the EGP region in the past 6 years (Sharma *et al.,* 2007). Since high temperatures also aggravate spot blotch severity (Nema and Joshi, 1973; de Lespinay, 2004; Sharma and Duveiller, 2004; Duveiller *et al.,* 2005) wheat yield losses in the EGP region could be due to increase in spot blotch epidemic over the recent years, which could curtail the benefits from progress recently achieved by local breeders toward incorporating resistance in locally adapted high yielding varieties. Hence, increased spot blotch severity due to

steadily changing climatic conditions would worsen the damage already caused by heat with the reduction of assimilates translocated to the grain. Likewise, climatic change in South Asia has been observed in terms of a significantly increasing number in cloudy and foggy days during winter months (Debi, 2003), which not only reduces solar radiation use but also contributes to prolonged hours of 100% relative humidity in crop canopy, an ideal situation for early establishment of spot blotch in wheat.

Global climate change especially increased CO_2 and temperature level (Pachauri and Reisinger, 2007; Watson, 2001) is thought to influence or change all the elements of a disease triangle i.e. host, pathogen and weather factors and their interactions (Anderson *et al.,* 2004; Burdon *et al.,* 2006; Legreve and Duveiller, 2010). There are enough indication that climate change could alter stages and rates of development of the pathogen, modify host resistance, and results in changes in the physiology of host-pathogen interactions (Coakley and Schrem, 1996). Climate change may affect disease epidemics and can alter spatial distribution of agro-ecological zones, habitats, distribution patterns of plant diseases (Chakraborty *et al.,* 2008, Chakraborty and Newton, 2011). Effect of changing environment on the pathogen characteristics such as frequency of generations and proportion of sexual reproduction and the rate of adaptation affected is not known (Garrett *et al.,* 2006). Predictions on how changes in climate will affect plant health requires knowledge on already observed effects of climate change on plant diseases, extrapolation from expert knowledge and experimental studies, and computer models. Identifying and quantifying impacts of climate changes on plant diseases is complex as both climate change and climate variability affect plant disease epidemics (Coakley and Scherm, 1996; Shaw and Osborne, 2011) as well as there is a great deal of uncertainty about the accurate climate forecast. Both climatic variability and climate change are relevant drivers of plant disease epidemics and expected to alter the synchrony between crop phenology and disease or pest patterns. Climate change in terms of elevated CO_2 and temperature is now a concern as the change may probably influence the occurrence, prevalence and severity of plant diseases as evidenced from number of observations, anticipated, or possible consequences on crop health worldwide (Chakraborty *et al*., 2008, Legreve and Duveiller, 2010). Changes in climate may modulate host susceptibility/resistance responses to pathogens (van

Maanen and Xu, 2003). Spot blotch (*Bipolaris sorokiniana*), once unknown or of minor importance, has become a serious problem in wheat.

Increasing trend in temperature during winter probably makes favourable situation for spot blotch in north western part. Appearance of the disease is influenced by weather factors especially temperature and high relative humidity. However, little is known about the importance of small changes in temperature under field conditions, or the biometeorological basis of responses to such changes. Perusal of literature indicated there has been no study in this direction. There is a need to identify and explain changes in disease scenario that may help to predict or assess regional vulnerability due to changes in order to generate information and knowledge to develop adaptation strategies, techniques and methodologies. The objective in the current chapter was to assess the changes in spot blotch epidemic components in wheat with reference to spot size, number, disease severity, incubation period and latent period under elevated CO_2 and temperature.

6.4. Materials and methods

All investigations were conducted at the laboratory of Plant Pathology Division and Phytotron facility, Indian Agricultural Research Institute (IARI) (28°38'23"N, 77°09'27"E, 228.61 m above mean sea-level), New Delhi, India.

6.4.1. Preparation of inoculum

B. sorokiniana was isolated from one heavily infected field of Institute's experimental farm was grown for 10 days in water agar plates at 24°C. Spore suspension was prepared by adding sterile distilled water and gently scrapping the media surface with a spatula. The suspension was strained through two layers of chess cloth and spore concentration was adjusted to 3×10^4 spores ml^{-1} (Shamim *et al.,* 2008) using haemocytometer (Neubauer improved, Superior Marienfeld, Germany). One drop of Tween-20 per 100 ml of suspension was added as wetting agent. Spore suspension was stored in -5°C for carrying out inoculation in the season.

6.4.2. Preparation of plants in Phytotron

For raising uniform seedlings, earthen pots (diameter 15 cm) were filled up with mixture of vermiculite and peat (1:1) and sown with seed of susceptible wheat variety (Agra local). Immediately after germination pots were shifted to growth chambers (Conviron, E15, Canada) under phytotron facility keeping uniform

irrigation (100 ml per pot twice in a week), light intensity (5-10 Klux), 12 h photoperiod and RH 60-70%.

6.4.3. Experimental treatments

Growth chambers were set with various levels of CO_2 and temperature in following manners:

Trt_1: ambient CO_2 – ambient temperature (360 ppm-22°C)

Trt_2: high elevated CO_2 – ambient temperature (550 ppm-22°C)

Trt_3: ambient CO_2 – elevated temperature (360 ppm-25°C)

Trt_4: high elevated CO_2 – elevated temperature (550 ppm-25°C)

6.4.4. Inoculation procedure

Inoculation was performed on thirty-day old plants having exposed under elevated conditions. The plants were sprayed uniformly by spore suspension of the pathogen (5 ml per pot) using hand atomizer. For making sufficient humidity required for infection, pots were covered with polyethylene bags immediately after inoculation (24 *h*) and observed for time of spot development.

6.4.5. Observation on spots development

Three inoculated leaves were randomly selected from each pot (9 leaves for each replication, 27 leaves for each treatment) and marked by marker for daily counting of spot number till reached maximum number (asymptote) after which there was no increase in spot number. Digital images of the selected leaves were taken at regular intervals through a digital camera (Sony, Japan) and spot size and number were counted through image analysis software (Assess 2.0). Thresholds for leaf colour were established in the HSI (Hue, Saturation and Intensity) colour space and image was segmented based on threshold values using classic panel for the counting of number of spots (Lakhdar, 2008).

Mean area of spots was evaluated by measuring of randomly 20 selected spots (as standard error for spot size stabilized at about 20-22 spots) in every infected leaf and whole leaf area also was measured and number of spots cm^{-2} of leaf area estimated. Percentage of necrotic spots on the wheat leaves was evaluated by multiplication of spots number per cm^2 of leaf area into the average area of necrotic spots.

For evaluation of latent period, immediately after symptom appearance, two infected leaves were selected from each pot (6 leaves for each replication, 18 leaves for each treatment) and selected leaves were cut into 2-3 pieces and placed on wet

and sterilized filter papers inside Petri plates for providing sufficient humidity required for sporulation. Petri plates were sealed by parafilm stripes and kept inside incubator with similar temperature (22 and 25°C) as fixed for exposure experiments. Wheat leaves inside Petri dishes were examined under binocular microscope (Olympus, CH20i, BIMF, Japan) every day and number of spots which had started sporulation was noted down till completion of sporulation on almost all spots. The time from inoculation up to sporulation of 50% of the estimated maximum number of spots was considered as LP_{50} and assessed by calculation of cumulative frequency and percentage evaluation using Microsoft Excel program.

6.4.6. Effect of elevated temperature on spot blotch in wheat under temperature gradient chambers

Chambers for controlled temperature was used to expose +1, +2, and +3°C along with ambient temperature. Temperature levels (+1, +2, and +3°C) above ambient temperature in the controlled chambers was maintained using auto-sensor and regulatory fan devices to keep temperature inside the chambers at desired levels. Nine varieties of wheat - C306, HD2285, HD2329, HD2932, HD2967, Kundan, PBW343, PBW550 and WR544 were sown in the four chambers in normal date of sowing in the gradient chambers prepared by Centre of Environmental Sciences and Climate Resilient Agriculture, IARI, New Delhi. Spot blotch severity was noted during the end of March following double-digit scale (*DD*, 00–99) (Saari and Prescott, 1975). The first digit (D_1) indicates vertical disease progress on the plant and the second (D_2) indicates severity measured in diseased leaf area and disease severity percentage was estimated as $\left(\frac{D_1}{9}\right) \times \left(\frac{D_2}{9}\right) \times 100$ (Sharma *et al.*, 2007).

Hourly weather data was recorded though Kestrel weather kits nearby chambers.

6.4.7. Data analysis

For assessment of mean number of spots cm^{-2}, mean area of spots and percentage of necrotic spots, analysis of variance (ANOVA) was used in completely randomized design (CRD) with 3 replications every one comprised/consisted of 3 pots with 5 seedlings in each pot. All data analyzed using SPSS ver.16.

For evaluating of incubation period (IP_{20}), the time duration between inoculation and 50% of spots appearance was considered as IP_{20} and calculated from cumulative frequency using Microsoft Excel program. Similarly for latent period (LP_{50}) the time duration from inoculation up to sporulation of 50% of the estimated

maximum number of spots was considered as LP_{50} estimated by calculation of cumulative frequency using Microsoft Excel program. Observed IP_{20} in the elevated exposure experiments was compared with the predicted IP_{20} estimated through the model developed for hourly IP_{20} completion rate established as *(0.002 Temp –0.03) {1 – exp [0.151(Temp – 36)]}.*

6.5. Results

6.5.1. Assessment of spots number, size and necrotic leaf area under elevated exposures

Typical spots with yellow colored halo appeared on inoculated leaves and by 10 days maximum symptoms expressed. After symptom appearance, gradually parts of leaves around necrotic spots were tended to yellowish color and changed into chlororotic parts particularly due to coalescence of spots. Epidemic parameters like number of spots cm^{-2} of leaf, mean spot area (mm^2) and percentage of necrotic area were found to change under elevated CO_2 and temperature treatments.

6.5.2. Effect of elevated CO_2 on wheat leaf area

Leaf area (cm^2) were found to increase significantly in plants exposed one month under elevated CO_2 (550 ppm) as compared to the plants in ambient CO_2 level (360 ppm) irrespective of temperature level (Fig.6.1). Plants exposed at 360 ppm CO_2 level with temperature 22 and 25°C did not show significant variation. Similarly, increase in CO_2 level at 550 ppm although there was significant increase in leaf area but did not show difference in leaf area whether exposed at 22 or 25°C. Therefore, temperature may not have direct role to affect leaf area at least in the range 22-25°C tested.

6.5.3. Spot number

Under elevated CO_2 level comparatively lower number of spot /cm^2 leaf area was noted than at ambient CO_2 level (Fig.6.2). However, temperature level 25°C found to increase spot number irrespective the level of CO_2 and till 10 days of observation trend in spot number did not vary (Fig. 6.4A).

6.5.4. Spot area

Spot area (mm^2) was found to increase significantly in plants exposed under higher levels of CO_2 (550 ppm) as compared to the plants in ambient CO_2 level (360 ppm). Plants exposed at CO_2 level 360 ppm with temperature 22°C did not show significant variation in spot size with the plants exposed at similar CO_2 level and temperature

25°C (Fig.6.3). With the increase in CO_2 level at 550 ppm although there was significant increase in spot area but at 25°C there slight decrease in number. The trend in spot area or size remained similar till 10 days of observation (Fig.6.4B). Therefore, temperature may not have significant role to influence spot area.

6.5.5. Incubation period

Incubation period (IP_{50}) estimated from the daily number of spot count was found to decrease significantly with the increase in temperature levels studied (Fig. 6.5). Apparently elevated CO_2 did not show marked variation however, at CO_2 level 550 ppm there was slight reduction in incubation period. Incubation period (IP_{20}) estimated based on the model for hourly rate of incubation period completion was found to be shorter at 25°C as compared to 22°C. However, the observed and predicted IP_{20} indicated that elevated CO_2 exposure did not have any effect on incubation period as they were at par corresponded well with the prediction done through incubation period model (Fig. 6.6).

6.5.6. Latent period

Latent period (LP_{50} - the time duration from inoculation up to sporulation of 50% of the spots) estimated from the daily count of sporulating spot found to decrease significantly as with the increase in temperature from 22 to 25°C (Fig.6.7). However, effect of elevated CO_2 did not appear to be significantly prominent.

6.5.7. Necrotic area produced by spots

Necrotic area (%) due to spot development was found to increase significantly with the increase in the level of CO_2 (Fig.6.8) and temperature indicating interaction between the effect of CO_2 and temperature. For individual treatments there was no change in trend in necrotic area till 10 days there was no significant departure from the trend (Fig. 6.9).

6.5.8. Effect of elevated temperature on spot blotch in wheat under temperature gradient chambers

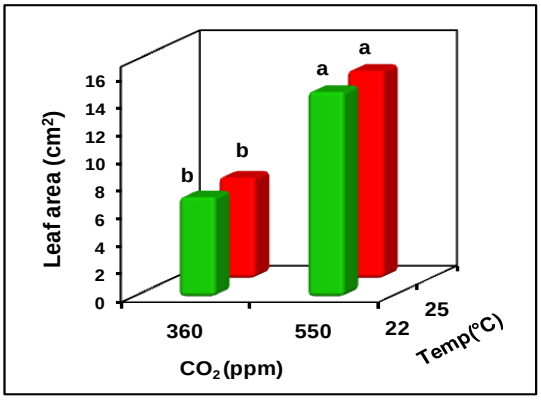
Out of nine cultivated wheat varieties (C306, HD2285, HD2329, HD2932, HD2967, Kundan, PBW343, PBW550 and WR544) grown in controlled temperature chambers with ambient, +1, +2 and +3, except C306 all were shown to be prone to higher spot blotch severity (Table 6.1) which was influenced by higher temperature. In chambers with elevated temperature, combined spot blotch severity taken for three levels of temperatures for all the varieties was 13-24 as compared to ambient where severity grade was 11-22. Disease severity in all chambers with elevated temperature was

shown marginal increase as compared to ambient temperature. Therefore, higher spot blotch severity in elevated temperature indicated the influence of higher temperature on the development for the pathogen.

Table 6.1: Spot blotch severity based on Saari and Prescott scale (1975) at milking stage* on wheat varieties noted in elevated temperature gradient chambers and ambient conditions as well as natural conditions during January-March 2013.

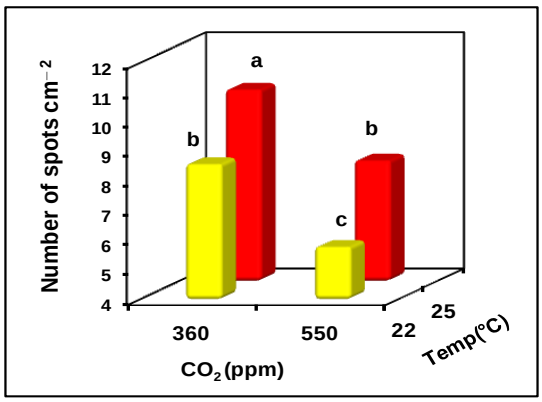
Wheat varieties	Spot blotch severity		
	Elevated condition (+ 1-3°C) inside chamber	Ambient condition inside chamber	Natural condition outside of chamber
C306	13	11	Trace
HD2285	24	12	11
HD2329	16	15	14
HD2932	15	Trace	11
HD2967	14	11	12
Kundan	25	22	11
PBW343	24	14	15
PBW550	24	15	15
WR544	22	11	12

*0-9 scale followed.



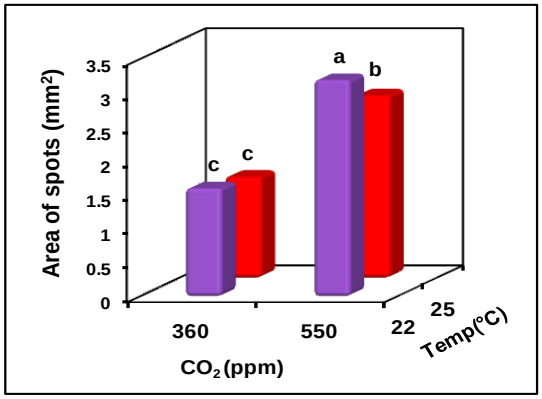
Treatment	leaf area (cm^2)
360-22	7.2 b
550-22	14.8 a
360-25	7.3 b
550-25	15 a
CD at 5%	0.776
CV (%)	3.89

Fig.6.1: Influence of different combined treatments of CO_2 and temperature (ambient and elevated levels) on wheat leaf area (with CD and CV in Table right side) after inoculation with *Bipolaris sorokiniana.*



Treatment	No.spots/cm^2
360-22	8.6 b
550-22	5.8 c
360-25	10.5 a
550-25	8.1 b
CD at 5%	0.737
CV (%)	5.09

Fig.6.2: Effects of different treatments of CO_2 and temperature (ambient and elevated levels) on number of spots cm^{-2} of leaf area (with CD and CV in Table right side) in wheat plants after inoculation with *Bipolaris sorokiniana*.



Treatment	Area of spots (mm^2)
360-22	1.6 c
550-22	3.2 a
360-25	1.5 c
550-25	2.7 b
CD at 5%	0.434
CV (%)	10.38

Fig. 6.3: Effects of different treatments of CO_2 and temperature (ambient and elevated levels) on area of spots (with CD and CV in Table right side) in wheat plants after inoculation with *Bipolaris sorokiniana*.

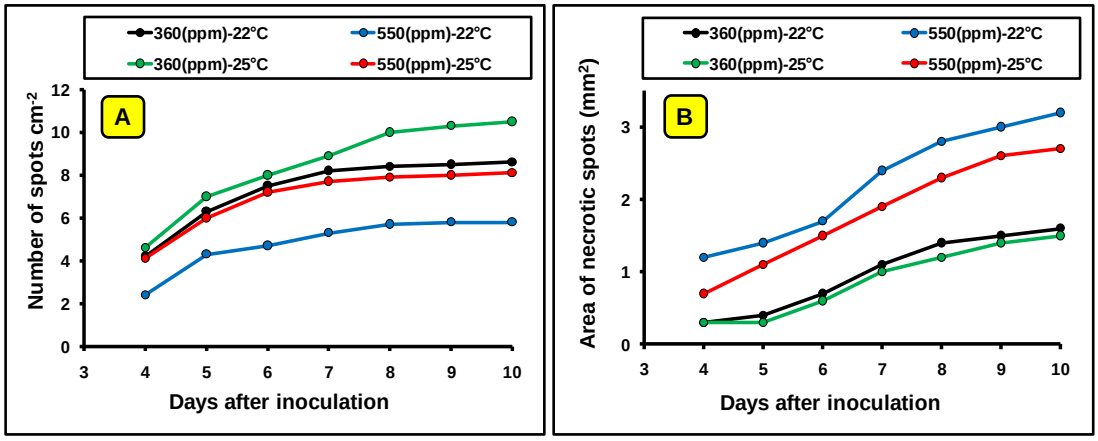
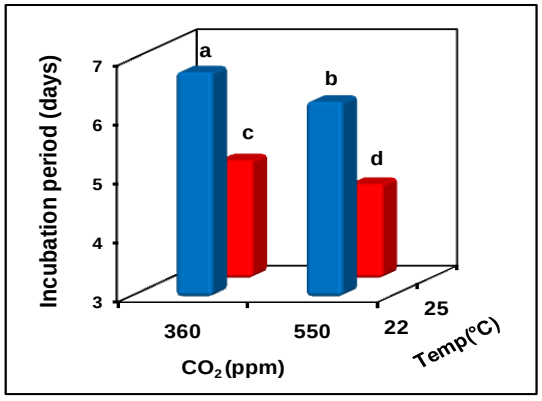


Fig. 6.4: Effect of different combined treatments of CO_2 (ppm) - temperature (°C) levels (ambient and elevated) on Number of spots cm^{-2} of leaf area (A) and average area of necrotic spots (B) in wheat leaves in different days after inoculation with *Bipolaris sorokiniana.*



Treatment	IP_{50}
360-22	6.8 a
550-22	6.3 b
360-25	5.0 c
550-25	4.6 d
CD at 5%	0.38
CV (%)	3.63

Fig.6.5: Influence of different combined treatments of CO_2 and temperature (ambient and elevated levels) on incubation period of spot blotch disease (with CD and CV in Table right side) in wheat after inoculation with *Bipolaris sorokiniana*.

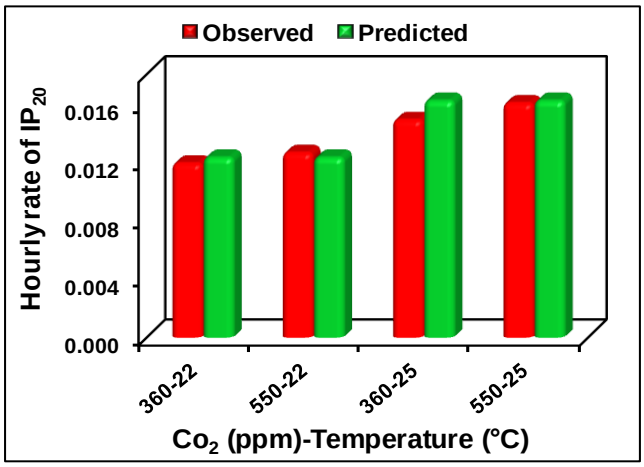
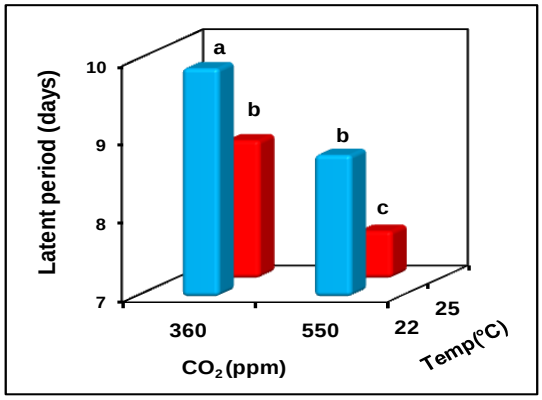
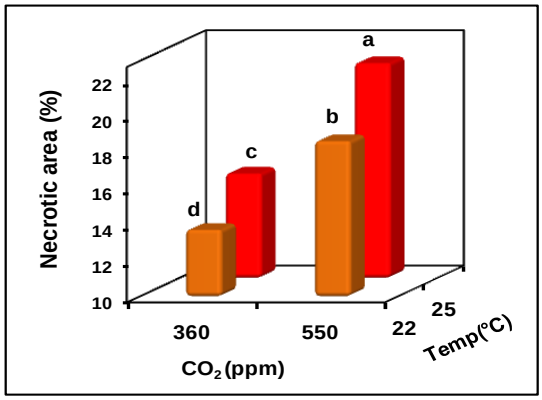


Fig.6.6: Observed and predicted hourly rate for IP_{20} completion in spot blotch disease of wheat in different combined treatments of CO_2 and temperature (ambient and elevated levels).



Treatment	LP_{50}
360-22	9.9 a
550-22	8.8 b
360-25	8.75 b
550-25	7.6 c
CD at 5%	0.555
CV (%)	3.44

Fig.6.7: Influence of different combined treatments of CO_2 and temperature (ambient and elevated levels) on latent period of spot blotch disease in wheat (with CD and CV in Table right side) after inoculation with *Bipolaris sorokiniana*.



Treatment	Leaf necrotic area (%)
360-22	13.7 d
550-22	18.6 b
360-25	15.8 c
550-25	21.9 a
CD at 5%	1.49
CV (%)	4.7

Fig. 6.8: Influence of different combined treatments of CO_2 and temperature (ambient and elevated levels) on percentage of necrotic area in wheat leaves (with CD and CV in Table right side) after inoculation with *Bipolaris sorokiniana*.

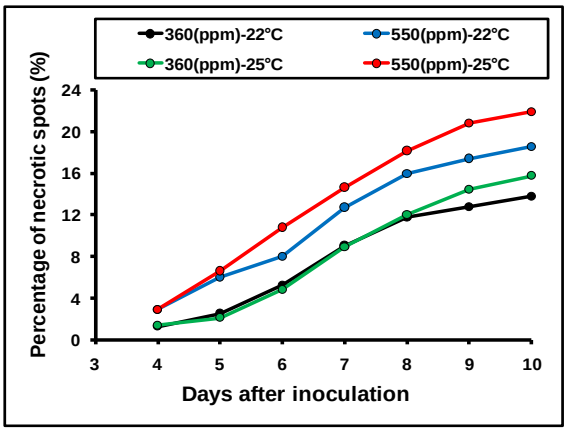


Fig.6.9: Effect of different combined treatments of CO_2 (ppm) - temperature (°C) levels (ambient and elevated) on percentage of necrotic spots (C) in wheat leaves in different days after inoculation with *Bipolaris sorokiniana.*

6.6. Discussion

Climate change especially rise in $CO_2$and temperature level is expected to affect both host and pathogen and thereby disease expression (Coakley and Schrem, 1996) due to alteration in host, pathogen and their interactions (Chakraborty and Newton, 2011). Change in epidemic features namely, primary infection rate, incubation /latent period and basic infection rate, disease progress curve, parameters for resistance and susceptibility enables to explain the impact of elevated exposure level. Both qualitative and quantitative estimation is required to explain the impact of climate change on host-pathogen interaction. Current study establishes the impact of elevated CO_2 and temperature in wheat with reference to spot blotch disease based on the changes on epidemic parameters, *i.e.*, number of spots cm^{-2} leaf area, mean spot area (mm^2), percentage of necrotic area, incubation period and latent period and percentage of necrotic area. Climate change due to increased level of CO_2 known to increase photosynthetic rate and changes on morphological features directly that might alter crop microclimate in favor of pathogen infection (Chakraborty and Newton, 2011). Together an increase in plant canopy size especially in combination with humidity, an increase in abundance and biomass can increase the size of pathogen population (Chakraborty and Datta, 2003; Pangga *et al.*, 2004). Through elevated CO_2 and temperature (+1.5°C) exposure in growth chamber we have noted increase in leaf area in wheat irrespective of temperature effect. In addition to leaf area increase we have observed reduction in stomata number, increase size of stomata and cuticular thickening under elevated CO_2 level (data not given). Waxy leaf surface and thicker cuticle may likely to reduce the efficiency of pathogen's entry due to increased surface hydrophobicity and epidermal barrier. Penetration of spot blotch pathogen into the host is reported both through cuticle and /or stomata (Kumar *et al.*, 2007).

Current observation, comparatively lesser number of spot /cm^2 leaf area under elevated CO_2 level than at ambient CO_2 level, indicates elevated CO_2 exposure probably reduces spot blotch pathogen entry in wheat. Chakraborty *et al.*, (2000) studied the effect of elevated level of CO_2 on *Colletotrichum gloeosporiodes* and showed delayed or reduced conidial germination, germ tube growth, appresorium production when inoculated onto susceptible tropical legume pasture *Stylosanthes scabra* cv. Fitzroy. It appears elevated CO_2 exposure of wheat plants might have

slightly impaired pathogen's entry as far as host surface interaction is concerned. Germination of *B. sorokiniana* spores on wheat leaves under elevated and natural conditions is to be compared whether the effect is due to CO_2 level rise. However, spot area (mm^2) was found to increase significantly in plants exposed under higher levels of CO_2 (550 ppm) as compared to the plants in ambient CO_2 level (360 ppm). It appears that although there might be little impairment in establishing the pathogen but its growth in relation to pathogenesis is not affected under elevated CO_2 level. Rising CO_2 has been reported to affect initial establishment of the pathogen on the host (Coakley and Schrem, 1996; Matros *et al.,* 2006) and increased fecundity and growth of some fungal pathogens (Hibberd *et al.,* 1996; Chakraborty *et al.,* 2000).

For spot blotch, although elevated CO_2 exposure indicated a slight decrease in incubation period or otherwise increased fecundity but it appears that the effect may be due to some indirect reason. In both biotrophs and necrotrophs, significant changes have been reported in the onset and duration of stages in the pathogen life cycle under elevated CO_2 and latent period was extended (Chakraborty *et al.,* 2000). Together an increase in plant canopy size especially in combination with humidity, an increase in abundance and biomass can increase the size of pathogen population (Chakraborty and Datta, 2003; Pangga *et al.,* 2004). But it is to be seen magnitude of plant canopy modification that could alter plant microclimate in favor of pathogen infection as far temperature and leaf wetness is concerned. However, elevated temperature has shown distinct effect on the pathogen growth as there have been changes in number and size of spot, incubation period, latent period and necrotic area due to spot development. Significant decrease in incubation and latent period due to increase in temperature is expected based on the incubation period model as higher temperature has relatively shorter period. Under elevated exposure the observed and predicted IP_{20} (through developed model) irrespective of CO_2 indicated that elevated exposure did not have any effect on incubation period as they were at par corresponded well with the prediction done through incubation period model. Increased spot blotch severity under temperature gradient (with +1, +2, and +3°C above ambient temperature) clearly supports the view that temperature effect on the disease expression is prominent. Therefore, direct effect of temperature rise on pathogen and host and probably indirect effect of changes in host are contributing to a definite increase in spot blotch development.

Prediction of spot blotch distribution in wheat under climate change scenario in Indo-Gangetic plains

7.1. Abstract

Spot blotch (*Bipolaris sorokiniana*) in wheat is one of the most important diseases in warmer areas of the world particularly in South Asia. Effect of incubation period (IP_{20} - time from start of infection to 20% spot appearance) were estimated for prediction of spot blotch infection under elevated temperature. A model for hourly IP_{20} completion rate established as $(0.002\ Temp - 0.03)\ \{1 - exp\ [0.151(Temp - 36)]\}$ could reasonably explain the rate of spot development for diurnal temperature fluctuations tested under natural infection in the field. Assessment of spot blotch favorable hours and sum of rate for IP_{20} completion under current situation indicated that eastern region of Indo-Gangetic plains is relatively more favorable during February-March than western region. Rise in temperature (0.5-3.0°C) due to climate change increased disease favorable hours and developmental rate during February and March, however, percentage of increase in February was more than in March and western plains were shown to be more sensitive to temperature rise. A criterion for monitoring of infection favorable period and a developmental rate has been proposed to develop a spot blotch forecasting system for scheduling protective fungicide applications as the severity of the disease is likely to increase further under climate change scenario.

7.2. Key words: Wheat, Spot blotch, Favorable hours, Incubation period, Climate change, Prediction, Distribution, Indo-Gangetic plains

7.3. Introduction

The Indo-Gangetic plains in India contributes nearly one third of the total food grain production of the country in which wheat (*Triticum aestivum* L.) is a major contributor. Several fungal diseases constrain wheat production. Spot blotch caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker is one of them.The disease is distributed worldwide and reported from South and East Asia (Saari, 1998), North and Latin America, Africa (Duczek and Jones-Flors, 1993), India (Joshi *et al.,* 2002), China (Chang and Wn, 1998) and Brazil (Mehta, 1998). The pathogen perpetuates both

externally as conidia and internally as mycelium in the seeds, as well as in infected crop residues, volunteer plants, secondary hosts and free dormant conidia in the soil (Reis, 1991). However, the role of infected seed as a primary source of inoculums appears to be important and according to Shaner (1981) as it is the main source of inoculums of leaf blight pathogens. About 25 million hectare of cultivated wheat area is affected worldwide by spot blotch (Van Ginkel and Rajaram, 1998). It has become a major production constraint in South Asia's intensive rice-wheat cropping systems and around 12 million hectare wheat area is affected (Nagarajan and Kumar, 1998; Ruckstuhl, 1998; Singh *et al.,* 1998) by spot blotch every year and yield losses in the tune of 20% to 80% were reported (Duveiller and Sharma, 2009) in this region. Few studies indicated that spot blotch infection in wheat is highly influenced by weather factors mainly temperature and high relative humidity (Singh *et al.,* 1997; Singh *et al.,* 1998; Mehta, 1998; Reis, 1991; Senthil, 2004). Disease response in the field can vary differently because they experience a larger number and a wider range of environmental cues (including variations in light quality, temperature and RH level). However, little is known about the importance of small changes in temperature under field conditions, or the biometeorological basis of responses to such changes. The disease is increasingly becoming a cause of concern particularly in the warm and humid environments of Indian sub-continent (Duveiller *et al.,* 2005) where the mean temperature of the coolest month is higher than 17.5˚C (Dubin *et al.,* 1998). More recently, spot blotch has also expanded into the cooler, non-traditional irrigated rice-wheat growing areas (Duveiller and Gilchrist, 1994; Sharma *et al.,* 2007). Since high temperatures aggravate spot blotch severity (de Lespinay, 2004; Sharma and Duveiller, 2004; Duveiller *et al.,* 2005) wheat yield losses in the region could be due to increase in temperature which resulted in spot blotch epidemic over the recent years (Sharma et al. 2007). Moreover, recent survey indicated that spot blotch spread to non-endemic areas in Pakistan (Shamim *et al.,* 2010) might be due to temperature rise. In Indian sub-continent wheat is already facing yield reduction due to terminal heat stress and increased spot blotch severity may aggravate further yield damage (Nagarajan, 2005; Juroszek and von Tiedemann, 2013).Climate change in South Asia not only increased the temperature but increased number of cloudy and foggy days during winter months also (Debi, 2003). It reduced solar radiation and lengthened the duration of high relative humidity resulting in early establishment of spot blotch in wheat (Sharma *et al.,* 2007). Due to global climate change, mean

global temperature rise is expected in the range 0.5-2.0°C by the end of this century (IPCC, 2013). This is likely to influence or change all the elements of a disease triangle i.e. host, pathogen and weather factors and their interactions (Anderson *et al.,* 2004; Burdon *et al.,* 2006; Legreve and Duveiller, 2010). Effect of changing environment on the pathogen characteristics such as frequency of generations and predictions on how changes in temperature are likely to influence disease severity are largely unknown. There are enough indication that climate change could alter stages and rates of development of the pathogen, modify host resistance, and results in changes in the physiology of host-pathogen interactions (Coakley and Schrem, 1996). This may also affect disease epidemics; alter spatial distribution over agro-ecological zones, habitats and distribution patterns of plant diseases (Chakraborty and Newton, 2011). Temperature rise has been predicted in the range 1.2-2.8°C in Indo-Gangetic plains and rainfall is expected to increase about 20% by the end of century (Kothawale *et al.,* 2010). A rise of temperature in the range of 0.5-0.8°C above pre-industrial era has been already reported (Rupa-Kumar *et al.,* 2006; Kothawale *et al.,* 2010; IPCC, 2013). Garrett *et al.,* (2006) has underlined the intricate interrelationships between plant disease and climate. Climate change is characterized by greater increases in minimum temperature than maximum temperature and therefore, improved understanding of the temperature response is needed to better quantify and reduce uncertainties in climate change impact assessments (Lobell and Ortiz-Monasterio, 2007). To produce risk prediction maps, CLIMEX model (Sutherst *et al.,* 2004) based on long-term climate data and downscaled general circulation models (GCMs) for global weather forecast have been used to assess the impacts of potential global climate change for diseases (Pivona and Yang, 2004; Salinari *et al.,* 2006). However, all these models do not provide specific weather information at the canopy level and thus provide only very gross estimates of conditions that affect plant and disease development. Hourly and daily estimates of weather data only could explain pathogen infection at canopy level and best suited for disease risk prediction (Gleason *et al.,* 1997). Therefore, impact of temperature rise on disease, based on pathogen infection at canopy level using hourly resolution weather data is more realistic than long term weather based estimation of disease risk.

Change in favorable conditions in relation to rise in temperature may be useful to assess the impact of climate change on the disease. Temperature induced

incubation and /or latent period models could describe developmental or generation rate of the pathogen on host (Madden *et al.,* 2007) as incubation period is generally characterized by a decrease in duration as temperature rises from the minimum to the optimum, then an increasing duration with higher temperatures (Analytis, 1977; Logan *et al.,* 1976; Pfender, 2001). Effect of changing environment on the pathogen characteristics such as frequency of generations and predictions on how changes in temperature will affect plant health requires knowledge on already observed effects of climate change on plant diseases, extrapolation from expert knowledge and experimental studies, and computer models (Garrett *et al.,* 2006; Savary *et al.,* 2011). Latent period completion rate for stem rust in tollgrass and fescue was found to increase gradually from T_{min} up to T_{opt} and then decline more rapidly to reach zero at the T_{max} or lethal temperature (Pfender, 2001). Therefore, effect of diurnal temperature fluctuation either below or above these threshold levels ($T_{min,}$ T_{max} and T_{opt}) could be related with incubation or latent period to reflect the development of the pathogen on host. Such temperature-based developmental rate model is also likely to facilitate temperature rise effect on diseases that may happen under climate change regime. A perusal of literature has indicated that there was no systematic study to establish any such models for forecasting spot blotch in wheat and simulate the impact of climate change on the disease in the region.

In the present communication, therefore, objective was to i) determine favorable conditions for spot blotch infection in wheat, ii) develop a model for incubation period, and iii) simulate the effect of temperature rise on spot blotch distribution under climate change scenario based on infection favorable conditions and developmental rate.

7.4. Materials and methods

7.4.1 Studied area and locations

Throughout Indo-Gangetic plains, 25 locations representing different microclimates in six states (Punjab, Haryana, Delhi, Uttar Pradesh, Bihar and West Bengal) were considered as point data.

7.4.2. Use of incubation period model

As IP_{20} nearly matched with the time of initial spot appearance so it was considered for simulation. Rate for incubation period completion was expressed as the reciprocal of incubation period and estimated as incubation period per hour as weather data

collected was hourly. Patterns of rate in different temperature were captured by the following equation (Based on paper 2):

Hourly rate of incubation period completion=(0.002T–0.03) {1–exp [0.151(T– 36)]}

Where *T* is the average temperature during the one hour period.

This rate was directly considered as the fraction of one incubation period completed per hour at the ambient temperature. The fractions were summed as temperature records received, and reaching the sum to 1 was indicating completion of one incubation period.

7.4.3. Assessment of favorable hours and rate of incubation period completion throughout Indo-Gangetic plains under ambient and elevated temperature

During the crop season, December 2012 and March 2013, hourly weather data were collected from automatic weather stations throughout Indo-Gangetic plains available in India Meteorological Department (IMD) website. For each hour, relative humidity (RH) was calculated based on this equation (Wanielista *et al.,* 1997):

$$RH = 100\ ((112 - 0.1\ T + T_D)/(112+0.9\ T))^{8}$$

Where *T* is temperature and T_D is dew point temperature.

7.4.4. Adjustment and correction of weather data in canopy level

During the time of analysis it was observed that in day time about 20 to 40% higher RH and about 1-3°C lower temperature are maintained as compared to RH and temperature data records at 3 m height in automatic weather stations. So, for estimation of spot blotch infection favorable hours, canopy RH and temperature assumed to be at least 20% higher and 1.5°C less respectively.

Addition of 1.5°C on ambient air temperature was considered as elevated temperature under climate change scenario. For comparison between months and locations, total monthly favorable hours (temperature 18-34°C and relative humidity 95% or above) were calculated and counted at ambient and elevated air temperatures (ambient air temperature + 1.5°C). Similarly, for month and location wise, sum of hourly rate for incubation period completion was calculated at ambient and elevated air temperatures during December to March 2013.

7.4.5. Generation of GIS maps for Indo-Gangetic plains

A base map was prepared with the point data using ArcGIS 10.0. Thematic maps of favorable hours and sum of rates were prepared using surface interpolation (IDW geostatistical analysis) for relative demarcation of spot blotch infection prone areas based on temperature and RH-duration for different locations and months.

To cover a wide of range of temperature rise (0.5-3.0°C) under climate change scenario, three locations viz., Cooch Behar (West Bengal), Darbhanga (Bihar) and Gurdaspur (Punjab) have been considered for estimation of favorable period and sum of rate for incubation period completion to compare regional trend.

7.4.6. Ground truth data

For ground truth observation on spot blotch severity, during the crop season 2012-13, thirteen locations across Indo-Gangetic plains (Gurdaspur, Kapurthala, Ferojpur, Karnal, Hissar, Panchkhula, Faizabad, Pilibhit, Ballia, Bhagalpur, Bhojpur, Darbhanga and Cooch Behar) were selected and disease severity was noted during the end of March following double-digit scale (*DD*, 00–99) (Saari and Prescott, 1975). The first digit (D_1) indicates vertical disease progress on the plant and the second (D_2) indicates severity measured in diseased leaf area and disease severity percentage was estimated as $\left(\frac{D_1}{9}\right) \times \left(\frac{D_2}{9}\right) \times 100$ (Sharma *et al.*, 2007).

7.5. Results

7.5.1. Number of favorable hours throughout Indo-Gangetic plains under ambient and elevated temperature

Based on ambient temperature and relative humidity profiles, it was observed that in December 2012 and January 2013 there was no sufficient period of favorable hours (18-34°C and RH $\geq$ 95%) as temperature was lower than favorable range although RH-duration requirement was fulfilled. However, for spot blotch development favorable hours were sufficiently prevailed during February-March 2013 due to seasonal rise in temperature. It appeared higher number of favorable hours prevailed in eastern plains (highest in Ballia in Uttar Pradesh and Darbhanga in Bihar) in comparison to locations in western plains (Table 7.1, Fig. 7.1 A and C).

For assessment of favorable hours under elevated temperature, 1.5°C was added over ambient temperature for all locations and favorable hour was estimated. In February and March 2013, with the addition of 1.5°C, the number of favorable hours was found to increase in all locations across the whole plains (Table 7.1, Fig. 7.1 B and D) with a gradient from eastern plains (higher) to western plains (lower) except for few locations. However, proportion of increase in favorable hours due to 1.5°C rise in temperature was found to be higher in February as compared to March and proportion of increase was predominant in western plains than in eastern plains

(Fig. 7.2). It appeared that effect of elevated temperature due to climate change on spot blotch infection may be more pronounced in February than in March.

Temperature rise in the range of 0.5-3.0°C for three locations across the studied area viz., Cooch Behar and Darbhanga (hotspot in eastern plains) and Gurdaspur (in western plains) indicated maximum proportion of increase in favorable hours during February was in Gurdaspur where as high as 238% increase was noted as compared to133-195% increase in Cooch Behar and Darbhanga (Fig.7.3A). However, in March, temperature rise of 0.5-3.0°C did not show marked increase in favorable hours, but in Gurdaspur, proportion of increase was 10-113% as compared to 8-52% in Cooch Behar and Darbhanga (Fig.7.3B). Therefore, it indicated that impact of temperature rise on the disease would likely to increase because of probable increase in pathogen favorable hours throughout Indo-Gangetic plains. Disease might be more detrimental in already favorable eastern plains due to further increase in favorable hours and disease incidence is likely to increase in western plains where current incidence is in lesser extent.

7.5.2. Prediction of incubation period completion rate throughout Indo-Gangetic plains under ambient and elevated temperature

During February 2013, under prevailing temperature (ambient), sum of hourly rate completion simulated was found to be comparatively lower throughout Indo-Gangetic plains obviously due to low temperature (Table 7.2). In eastern plains (Cooch Behar, Darbhanga, Bhojpur, Siwan, Bhagalpur, Ballia and Deoria) sum of rates was in between 2-3 as compared to 0.7 in Gurdaspur located in western plains (Fig. 7.4 A). Under elevated conditions (+1.5°C), sum of rates was found to increase in the range of 3-4 in Bihar and West Bengal of eastern plains as compared to 1-2 in Punjab of western plains. Locations in the mid positions had sum of rates in intermediate range (Fig. 7.4 B).

Table 7.1: Number of favorable hours (Temperature ≥ 18°C and RH ≥ 95%) for spot blotch disease development inside wheat plants canopy throughout Indo-Gangetic plains under ambient and elevated temperature conditions (climate change scenario: +1.5°C) during February and March 2013.

State	Location	Total number of favorable hours			
		February		March	
		Ambient temperature	Elevated temperature	Ambient temperature	Elevated temperature
Punjab	Gurdaspur	34	69	116	169
	Kapurthala	50	93	156	217
	Ferojpur	17	37	95	148
	Patiala	54	100	162	233
	Nawanshahar	62	107	174	253
Haryana	Fatehabad	109	148	145	203
	Karnal	104	150	128	209
	Hissar	32	56	92	126
	Panchkula	50	88	179	246
	Chandigarh	31	57	80	125
Delhi	New Delhi	90	119	172	245
Uttar Pradesh	Faizabad	46	82	119	174
	Pilibhit	44	79	119	154
	Aligarh	47	98	99	146
	Lucknow	87	128	252	298
	Jaunpur	40	77	121	156
	Deoria	78	113	218	266
	Ballia	167	224	269	296
	Jhansi	8	15	10	18
Bihar	Darbhanga	165	278	348	423
	Nawada	81	100	85	114
	Bhagalpur	131	193	174	223
	Siwan	122	205	171	237
	Bhojpur	131	197	181	212
West Bengal	Cooch Behar	67	126	225	298

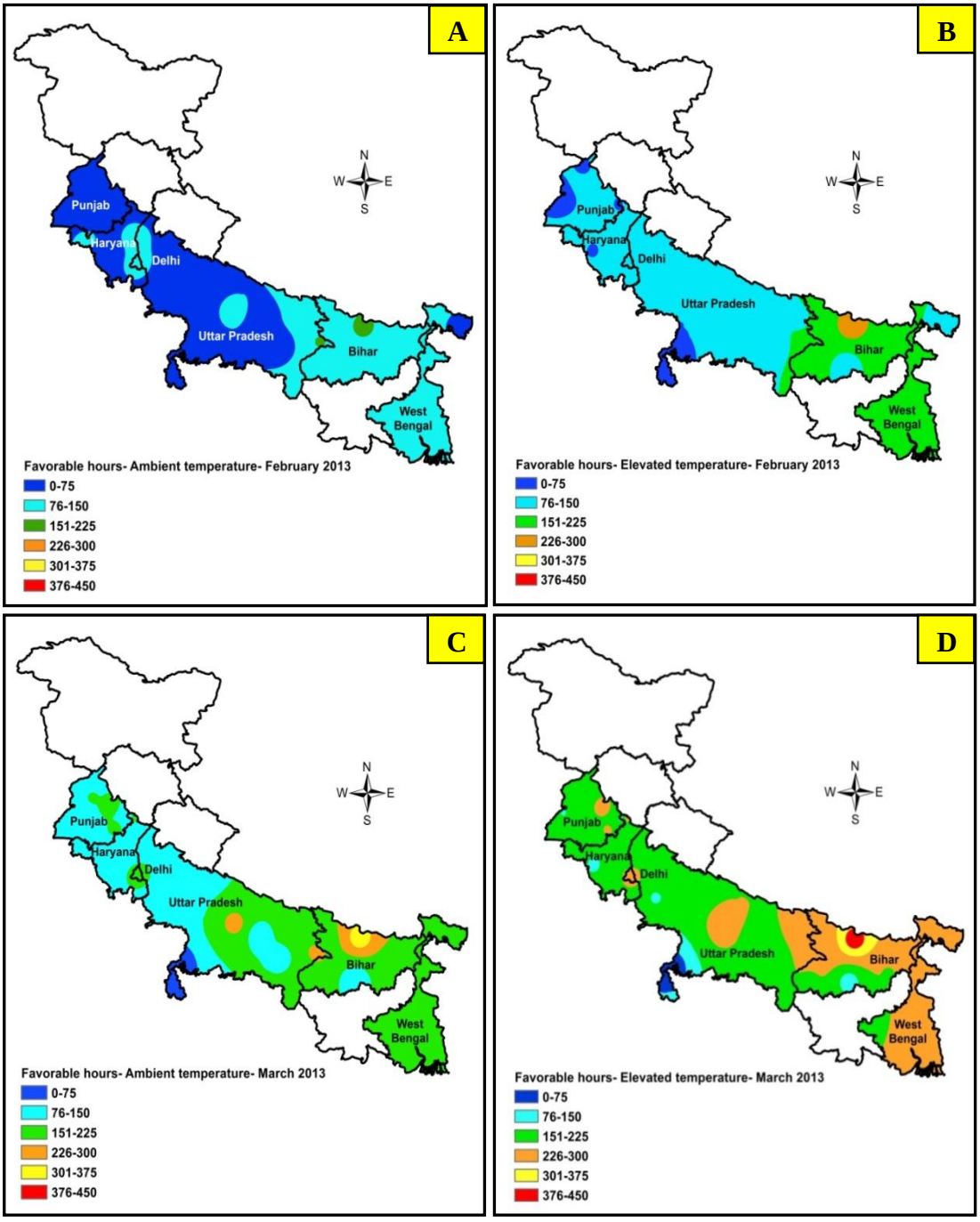


Fig. 7.1: Geographical distribution of monthly favorable hours for spot blotch infection in wheat throughout Indo-Gangetic plains during February-March 2013 under ambient temperature (A and C) and elevated temperature (B and D).

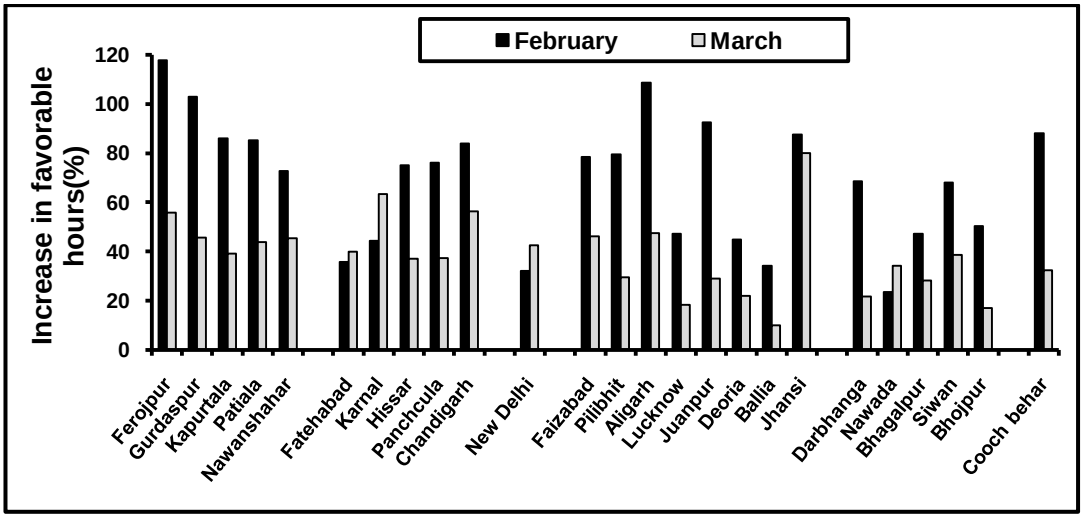


Fig. 7.2: Percent increase in number of favorable hours for spot blotch development in wheat due to increase in temperature (climate change scenario: +1.5°C) throughout Indo-Gangetic plains during February and March 2013.

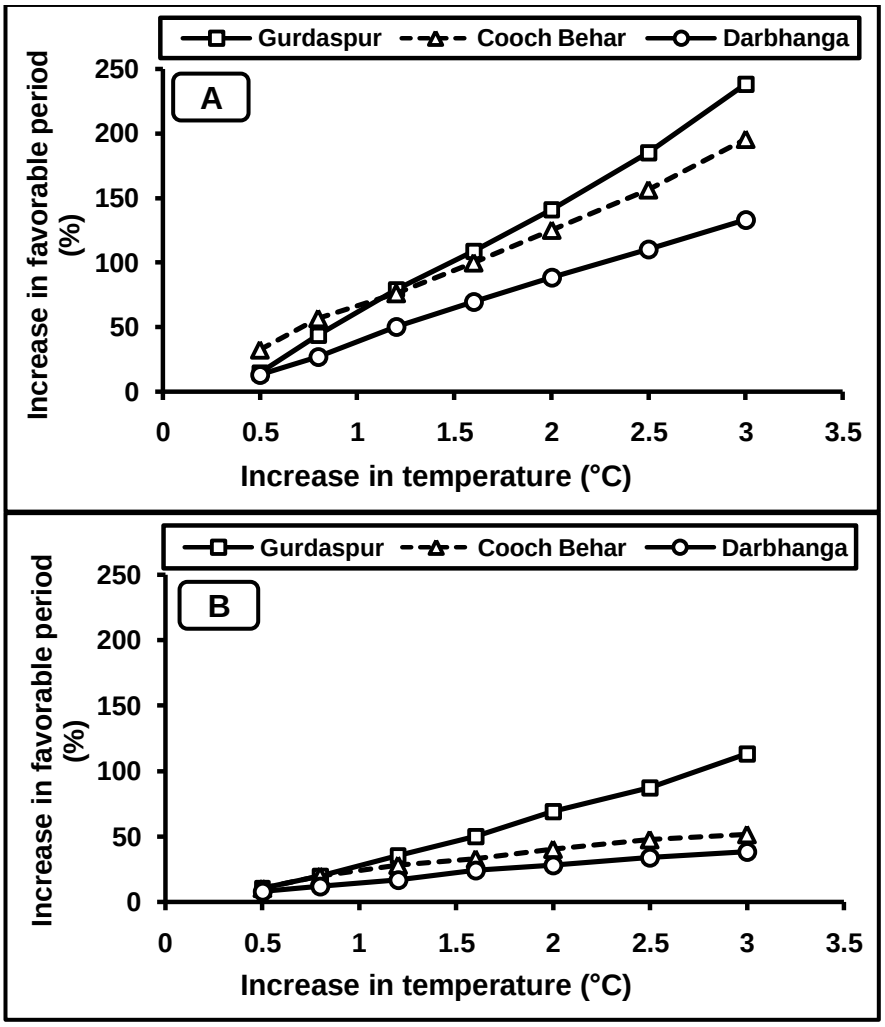


Fig. 7.3: Percentage of increase in monthly favorable period for spot blotch infection of wheat due to different levels of elevated temperature in February (A) and March (B) for three locations in Indo-Gangetic Plains.

Due to seasonal increase in prevailing ambient temperature during March 2013, sum of rates increased in the range of 5-8 in eastern plains whereas in western plains the sum of rates was 3-5 (Fig. 7.4 C), as well as with addition of 1.5°C over ambient conditions, sum of rates was noted to be in the range of 6-8 in the same locations of eastern plains whereas in western plains the sum of rates was 4-6 (Fig. 7.4 D). However, percentage of average increase in sum values was much higher during February than in March (Fig. 7.5). Therefore, temperature rise due to climate change would likely to favor earlier and faster development of spot blotch than the current situation seen throughout Indo-Gangetic plains.

Percentage of increase in sum of rates under temperature rise of 0.5-3.0°C during February-March for three locations viz., Cooch Behar, Darbhanga and Gurdaspur, had indicated a distinct trend in regional variation. In February, maximum proportion of increase in incubation period completion rate was in Gurdaspur, where 26-221% increase in comparison to 8-69% increase noted in Cooch Behar and Darbhanga (Fig. 7.6A). However, in March, temperature rise due to climate change did not show marked increase in sum of rates, but in Gurdaspur degree of increasing trend was prominent as compared to Cooch Behar and Darbhanga (Fig.7.6B).

Table 7.2: Sum of hourly rates for incubation period (IP_{20}) completion during February and March 2013 on wheat canopy level under ambient and elevated temperature (climate change scenario:+1.5°C) throughout Indo-Gangetic plains.

State	Location	Sum of hourly IP_{20} rate			
		February		March	
		Ambient temperature	Elevated temperature	Ambient temperature	Elevated temperature
Punjab	Gurdaspur	0.395	0.754	3.18	4.14
	Kapurtala	0.513	0.966	3.5	4.39
	Ferojpur	0.614	1.024	3.83	4.69
	Patiala	0.738	1.640	4.45	5.53
	Nawanshahar	0.803	1.928	4.71	5.79
Haryana	Fatehabad	0.594	1.056	3.74	4.66
	Karnal	0.778	1.243	4.37	5.41
	Hissar	1.005	1.607	4.51	5.42
	Panchkhula	0.983	1.587	4.71	5.83
	Chandigarh	0.941	1.612	4.73	5.83
Delhi	New Delhi	1.215	1.952	5.2	6.3
Uttar Pradesh	Faizabad	1.244	1.964	5.54	6.43
	Pilibhit	1.590	2.311	6.19	7.18
	Aligarh	1.395	2.079	6.44	7.29
	Lucknow	1.792	2.430	6.8	7.63
	Juanpur	1.840	2.518	6.83	7.56
	Deoria	2.043	2.780	6.89	7.76
	Ballia	2.154	3.000	7.2	7.97
	Jhansi	2.370	2.795	7.4	7.85
Bihar	Darbhanga	2.411	3.319	5.73	6.64
	Nawada	2.502	3.224	5.74	6.36
	Bhagalpur	2.393	3.212	6.44	7.21
	Siwan	2.557	3.470	6.44	7.45
	Bhojpur	2.534	3.462	7.18	8
West Bengal	Cooch Behar	3.048	3.800	6.2	7.25

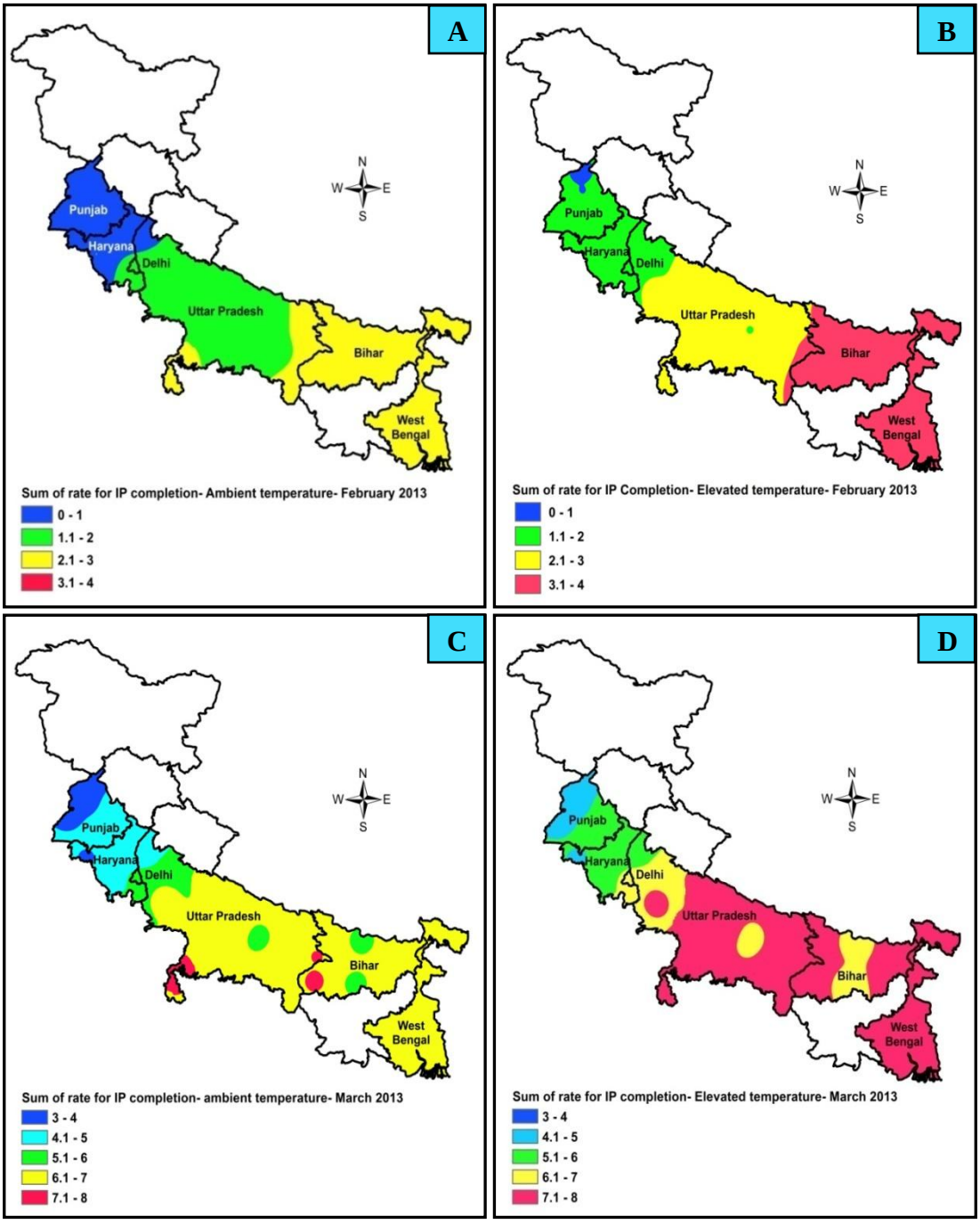


Fig. 7.4: Geographical distribution of incubation period completion rate (as monthly sum) for spot blotch development in wheat throughout Indo-Gangetic plains during February-March 2013 under ambient temperature (A and C) and elevated temperature (B and D).

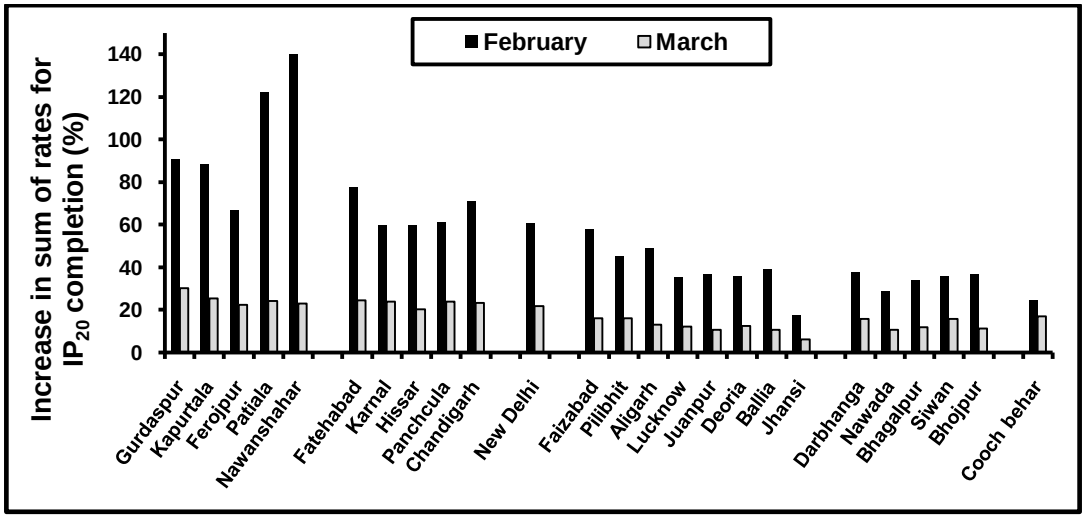


Fig. 7.5: Percent increase in sum of rates for incubation period completion for spot blotch development in wheat due to increase of temperature (climate change scenario: +1.5°C) throughout Indo-Gangetic plains during February and March 2013.

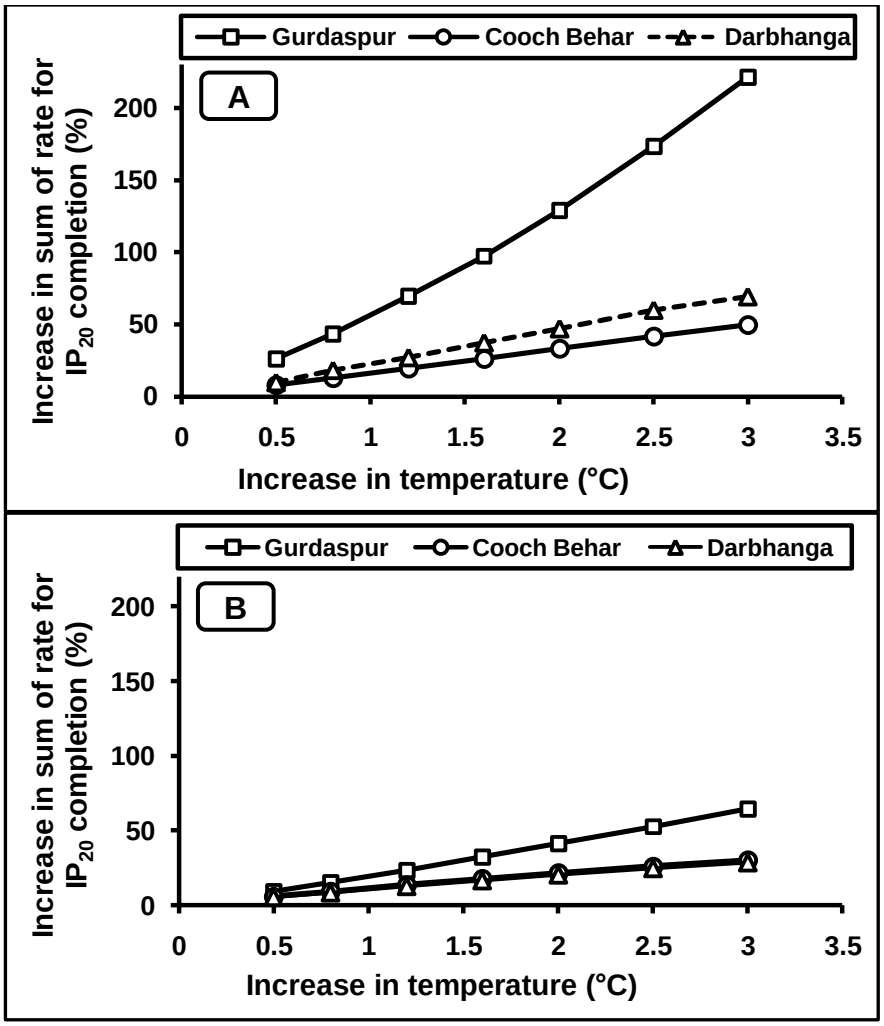


Fig. 7.6: Percentage of increase in monthly sum of incubation period completion rate for spot blotch development in wheat due to different levels of elevated temperature in February (A) and March (B) for three locations in Indo-Gangetic Plains.

7.5.3. Ground truth Data

Ground truth information collected in terms of spot blotch severity (double-digit) from several locations viz., Gurdaspur, Kapurthala, Ferojpur, Karnal, Hissar, Punchkhula, Faizabad, Pilibhit, Ballia, Bhagalpur, Bhojpur, Darbhanga and Cooch Behar, indicated the trend of higher severity in eastern plains as compared to lower severity in western plains (Table 7.3). Comparatively higher temperature in eastern plains was reflected with higher sum of rates for incubation period than in western plains. It became evident that temperature rise would likely to increase spot blotch favorable hours and developmental rate throughout Indo-Gangetic plains. In eastern plains currently existing favorable period as well as rate of incubation period would likely to increase further thereby disease severity may be escalated further in the region. Higher proportion of increase both in favorable hours and rate in western plains indicated likely much higher degree of spot blotch as that of now where the disease is less severe than the eastern plains. Therefore, elevated temperature is likely to incite earlier infection and likely increase in the length of congenial conditions together may escalate further disease severity throughout Indo-Gangetic plains.

Table 7.3: Spot blotch disease severity percentage in wheat based on double-digit score (Sharma *et al.,* 2007) for several locations in Indo-Gangetic plains during end of March 2013.

State	Location	Disease severity%
Punjab	Gurdaspur	30.1
	Kapurthala	35.5
	Ferojpur	40.1
Haryana	Karnal	56.2
	Hissar	57.3
	Panchkhula	56.1
Uttar Pradesh	Faizabad	67.1
	Pilibhit	66.2
	Ballia	77.2
Bihar	Bhagalpur	66.4
	Bhojpur	78.2
	Darbhanga	76.2
West Bengal	Cooch Behar	77.4

7.6. Discussion

Criteria for spot blotch infection and developmental rate model have been worked out to explain spot blotch development in wheat under climate change scenario. Current finding establishes the fact that occurrence of infection favorable temperature and RH-duration with a specified range in crop canopy facilitates spot blotch infection. Once infection is occurred subsequent progress of the disease is largely dependent on temperature. It has been evident that for spot blotch infection temperature rise both due to seasonal rise and climate change plays important role as duration of infection favorable conditions as well as rate of incubation period completion otherwise symptom expression is significantly influenced by change in temperature.

Based on infection favorable conditions, appearance of the disease and extent of severity observed in time and locations across whole Gangetic plains could be explained in relation to temperature. It has been possible to explain spot blotch appearance in the field as the disease was noted only when duration of favorable period exceeded the minimum duration requirement. Therefore, favorable conditions described could be used as criteria for determining approximate time of infection in the field. Since canopy favorable period could explain spot blotch infection accurately than the favorable period counted based on weather data recorded at 2 m height therefore, for prediction of spot blotch infection correction of weather data at canopy level is required.

Temperature has greatly influenced spot blotch development as the number of spots increased with increase in temperature. Direct effect of increasing temperature on multiplication and sporulation rate of the pathogen is very well known *in vitro* and *in vivo* (Duveiller *et al.,* 1998) and higher temperature favored spot blotch severity (de Lespinay, 2004).

Effect of temperature rise on spot blotch favorable period and developmental rate has been used for forecasting the future spot blotch scenario under climate change regime. Spatial pattern of spot blotch severity throughout Indo-Gangetic plains could be explained based on favorable period and hourly rate of incubation period completion under ambient conditions. Comparatively higher values of favorable hours and rate of incubation period completion in most locations of eastern plains has corresponded well with higher disease severity noted in eastern plains in

comparison to western plains. It is also noted that in the hotspots of eastern plains infection may start even early indicating availability of longer favorable period for the region. Under climate change scenario, temperature rise in North Indian plains has been predicted in the range 0.5-2.0°C and rainfall pattern to increase about 20% by the end of century (Kothawale *et al.,* 2010; IPCC, 2013). Adding 0.5-3.0°C over current ambient temperature, prediction of higher level of severity has been reflected. In the present study, although moisture criteria has not been considered in the assessment of temperature impact, but based on increased rainfall forecast in future for the region, it is quite logical to believe higher level of spot blotch severity as moisture level is likely to be increased than the current level. Global forecast on temperature is not uniform as land surface temperature rise was shown to have regions with lower than normal temperature also (Watson, 2001). However, for prediction of developmental rate, the model is robust enough to capture effects of either increase or decrease in temperature. Simulation of spot blotch favorable period and developmental rates due to elevated temperature has indicated February might be the most critical month when both rates and favorable hours has shown to increase as compared to March. Our analysis has indicated that favorable hours in February is likely to get increased remarkably (based on % increase) due to addition of 1.5°C over the ambient temperature as minimum temperature gets increased. However, in March, addition of 1.5°C has shown only marginal increase in favorable hours and rates although seasonal temperature goes up. Climate change due to increased level of CO_2 directly known to increase photosynthetic rate and changes on morphological features that might alter crop microclimate in favor of pathogen infection (Chakraborty and Newton, 2011). On the other hand, increased temperatures affect reproductive processes more than they affect photosynthesis and vegetative growth. However, measurable difference or changes in host to favor or disfavor pathogen infection directly or indirectly is an intricate issue. Through elevated CO_2 and temperature (+1.5°C) exposure in growth chamber we have noted increase in leaf area in wheat irrespective of temperature effect and increase in spot size and necrotic area (%) as well as decrease in incubation and latent period. Therefore, direct effect of temperature rise on pathogen and host and probably indirect effect of changes in host are contributing to a definite increase in spot blotch development. Observation on increased spot blotch severity recorded during six seasons in South Asia on a set of local commercial cultivars along with increase in night temperature has been an

apprehension in wheat productivity of region (Sharma *et al.*, 2007). In Indian plains, temperature has been continuously shown to increase as the region has already experienced 0.5-0.8°C temperature rise above pre-industrial era (Rupa- Kumar *et al.*, 2006; Kothawale *et al.*, 2010). Recent survey has indicated that spot blotch has gone to non-conventional areas like Pakistan (Shamim *et al.*, 2010) might be the cause of temperature rise. Therefore, current rising trend in spot blotch occurrence reasonably could be ascribed due to rise in temperature.

Overall, a criteria for monitoring of spot blotch infection favorable conditions has been determined considering whole range of pathogen growth on wheat and a temperature based rate of incubation period completion model has been established to develop a forecast system for scheduling protective sprays. Further, an analysis technique for the assessment of future disease risk under climate change scenario has been proposed which could be used as an instance for other diseases.

CHAPTER-VIII GENERAL DISCUSSION

Spot blotch, caused by *Bipolaris sorokiniana* is an emerging problem in wheat in warmer part of South Asia. Based on the current distribution of spot blotch across the globe, it is increasing being felt that temperature rise due to global warming is may be an important cause of disease rise. Although temperature and relative humidity or leaf wetness are reported to be the influential parameters for infection, but the infection possibilities under a range of factors have never been considered. Little is known about the importance of small changes in temperature under field conditions, or the biometeorological basis of responses to such changes. In the present investigation criteria for spot blotch infection for prediction of the disease has been developed and developmental rate model have been worked out to explain spot blotch development in wheat under climate change scenario. Current finding establishes the fact that occurrence of infection favorable temperature and RH-duration with a specified range in crop canopy facilitates spot blotch infection. Once infection is occurred subsequent progress of the disease is largely dependent on temperature. It has been evident that for spot blotch infection temperature rise both due to seasonal rise and climate change plays important role as duration of infection favorable conditions as well as infection cycle is significantly influenced by change in temperature.

An infection model based on temperature and high RH-duration has been developed which makes the basis forecasting for the disease. Infection model for fungal diseases is one of the most critical components for disease forecasting as infection process is usually limited by duration of RH or surface wetness in most terrestrial environments (Magarey *et al.*, 2005; Madden *et al.,* 2007). The model assumes presence of sufficient inoculum in the field and infection favorable temperature and RH-duration prevailed to establish the spot blotch infection. Temperature is one of the most important components for spot blotch development (Singh *et al.,* 1998; Senthil, 2004) but possibility of wider range for infection is necessary to explain infection under field conditions. Temperature behavior has been characterized as unimodal curve for fixing cardinal temperatures that accommodates the possible range to explain infections under field conditions.

Duration of high relative humidity, in addition to temperature, is the second important factor for spot blotch infection. Available information in the literature indicated either leaf wetness more than 18 h with temperature 18° to 32°C (Reis, 1991) or continuous rain for 5-6 days followed by warmer temperature (20°-30°C) triggers rapid development of spot blotch (Mehta, 1998). However, none of them considers entire dimension of moisture requirement necessary for infection, hence, a RH response curve explaining the infection process could not be developed based on such observation.

In present findings, RH response behavior on spot density has been worked in details to explain all variations and representative response curves, which is necessary for prediction of the disease, has been established. Based on the response curves, daily value of infection index may be predicted from the duration of RH and the average temperature prevailed on a particular day. Daily infection index may be used as the likelihood of spot blotch infection and its progress could be forecast provided availability of accurate weather forecast data.

The interaction of temperature and RH-duration in relation to spot blotch infection has been established. None of the literature has yet focused on this critical aspect of infection process. Hence, our study has generated important and valuable information in this regard for the first time. Maximum spot blotch infection index, asymptote (Y_{max}) and minimum time lag (m) is influenced by temperature. It has been realized from the relationship curve between m and temperature, that RH-duration requirement for spot blotch infection had decreased with increase in temperature up to 29°C while it had increased afterwards till 34°C. Since higher RH-duration was required for spot blotch development at temperature below or above 29°C, therefore, the fitted line was realistic and thus the relationship worked out is reliable to explain spot blotch infection. Through adjustment after field validation, it may be stated as that occurrence of infection favorable temperature (18-34°C) and RH-duration (15 *h* or above) in crop canopy facilitates spot blotch infection and once infection takes place subsequent progress of the disease is largely dependent on temperature. Therefore, it was possible to draw conclusions about the biological relevance of the shape of the response surface even though parameter estimates were derived from the controlled environment data.

Validation of the model under field condition indicated that canopy weather data is more reliable than the available weather data (recorded at 2 m height) for

prediction of accurate spot blotch infection. However, acquisition of canopy level weather data is practically not feasible through normal weather data recording devices. Hence, data on canopy weather conditions especially for temperature and RH-duration should be obtained after adjustment of differences between weather data available at 2 m height and at canopy level. Spot blotch has been also called as foliar blights as *Alternaria triticina* and *Pyrenophora repentis-tritci* are also to be associated with the disease as complex. The model does not consider the weather factors for these pathogens.

Temperature has greatly influenced spot blotch development as the number of spots increased with increase in temperature. Direct effect of increasing temperature on multiplication and sporulation rate of the pathogen is very well known *in vitro* and *in vivo* (Duveiller *et al.,* 1998) and higher temperature favored spot blotch severity (de Lespinay, 2004). In the present study, incubation period model for development of 20% spots from the start of infection has been considered and rate of incubation period completion otherwise generation rate has been established in relation to temperature. The valid use of rate summation methods to predict development under fluctuating temperatures assumes that the order in which different temperatures and RH-duration occur does not affect development (Hau *et al.,* 1985). The violation of this assumption may not lead to large errors in predicted developmental rate because, under field conditions, the fungus is unlikely to be subjected to such high temperatures or scanty RH-duration for a prolonged period in its early developmental stages. Thus, the model has revealed overall effect of temperature on spot blotch density after the initial infection took place. Quadratic model for incubation period could describe the characteristics decrease in duration in spot blotch development as temperature rises from the minimum to the optimum and increase in duration with higher temperatures. Linear quadratic models have been used to describe latent period behavior in barley rust (*Puccinia hordei*) (Teng *et al.,* 1980), alfalfa rust (Webb and Nutter, 1997) and *Colletotrichum* spp infecting strawberry fruit (King *et al.,* 1997) as they could explain the characteristics pattern of increase or decrease in developmental rate due to change in temperature. Pattern of incubation period for spot blotch development is not affected by RH-duration for infection. It is evident that RH-duration is essential only for establishment of infection on leaf surface and for development of symptoms temperature plays important role. Model for rate of incubation period completion or otherwise

developmental or generation rate in field testing has shown to capture the effect of hourly temperature in completion of a fraction of incubation period. The fraction or rate of incubation period completion is relevant to estimate the effect of diurnal temperature fluctuations on spot blotch development as the weights of temperature fluctuations around T_{min}, T_{opt} and T_{max} are taken into account. Thus the model could allow estimating the effect of temperature rise due to climate change. Hourly estimate of incubation-period progress can be estimated through the model as it is easy to fit and to apply to temperature data in a datalogger output. Other models (Analytis, 1977; Logan *et al.*, 1976) proposed for developmental rate are not straightforward as they require transformation of data (Pfender, 2001).

Climate change especially rise in CO_2 and temperature level is expected to affect both host and pathogen and thereby disease expression (Coakley and Schrem, 1996) due to alteration in host, pathogen and their interactions (Chakraborty and Newton, 2011). Change in epidemic features namely, primary infection rate, incubation /latent period and basic infection rate, disease progress curve, parameters for resistance and susceptibility enables to explain the impact of elevated exposure level. Both qualitative and quantitative estimation is required to explain the impact of climate change on host-pathogen interaction.

Current study establishes the impact of elevated CO_2 and temperature in wheat with reference to spot blotch disease based on the changes on epidemic parameters, *i.e.*, number of spots cm^{-2} leaf area, mean spot area (mm^2), percentage of necrotic area, incubation period and latent period and percentage of necrotic area. Climate change due to increased level of CO_2 known to increase photosynthetic rate and changes on morphological features directly that might alter crop microclimate in favor of pathogen infection (Chakraborty and Newton, 2011). Together an increase in plant canopy size especially in combination with humidity, an increase in abundance and biomass can increase the size of pathogen population (Chakraborty and Datta, 2003; Pangga *et al.*, 2004). In wheat, increased temperatures affect reproductive processes more than they affect photosynthesis and vegetative growth. No measurable differences were found in photosynthetic rates per unit flag leaf area or on whole-plant basis in the temperature range from 15 to 35°C. Through elevated CO_2 exposure in growth chamber we have noted increase in leaf area in wheat irrespective of temperature effect. In addition to leaf area increase we have observed reduction in stomata number, increase size of stomata and cuticular thickening under

elevated CO_2 level (data not given). Waxy leaf surface and thicker cuticle may likely to reduce the efficiency of pathogen's entry due to increased surface hydrophobicity and epidermal barrier. Penetration of spot blotch pathogen into the host is reported both through cuticle and /or stomata (Acharya *et al.,* 2011). In the present observation, comparatively lesser number of spot /cm^2 leaf area under elevated CO_2 level than at ambient CO_2 level, indicates CO_2 exposure probably reduces pathogen entry in wheat. Chakraborty *et al.,* (2000) studied the effect of elevated level of CO_2 on *Colletotrichum gloeosporiodes* and showed delayed or reduced conidial germination, germ tube growth, appresorium production when inoculated onto susceptible tropical legume pasture *Stylosanthes scabra* cv. Fitzroy. It appears elevated CO_2 exposure of wheat leaves might have slightly impaired pathogen's entry as far as host surface interaction is concerned. Confirmatory evidence is required whether reduced spore germination on wheat leaves under elevated CO_2 level is a possible reason for reduced spot number. However, spot area (mm^2) was found to increase significantly in plants exposed under higher levels of CO_2 (550 ppm) as compared to ambient CO_2 level (360 ppm). It appears that although there might be little impairment in establishing the pathogen but its growth in relation to pathogenesis is not affected under elevated CO_2 level. Rising CO_2 has been reported to affect initial establishment of the pathogen on the host (Coakley and Schrem, 1996; Matros *et al.,* 2006) and increased fecundity and growth of some fungal pathogens (Hibberd *et al.,* 1996, Chakraborty *et al.,* 2000). For spot blotch, although elevated CO_2 exposure indicated a slight decrease in incubation period or otherwise increased fecundity but it appears that the effect may be due to some indirect reason. In both biotrophs and necrotrophs, significant changes have been reported in the onset and duration of stages in the pathogen life cycle under elevated CO_2 and latent period was extended (Chakraborty *et al.,* 2000). Together an increase in plant canopy size especially in combination with humidity, an increase in abundance and biomass can increase the size of pathogen population (Chakraborty and Datta, 2003; Pangga *et al.,* 2004). But it remains to be seen whether plant canopy modification under elevated CO_2 really could alter plant microclimate in favor of pathogen infection as far as temperature and leaf wetness is concerned. Elevated temperature has shown distinct effect on the pathogen growth as there have been changes in number and size of spot, incubation period, latent period and necrotic area due to spot development. Significant decrease in incubation and latent period due to increase in temperature is

expected based on the incubation period model as higher temperature has relatively shorter incubation or latent period. Comparison of observed and predicted incubation period (through developed model) for two levels of temperature (22 and 25°C) indicated elevated CO_2 exposure did not have significant role in incubation or latent period reduction. Strong influence of temperature is further justified with the observation in gradient temperature chambers where increased level of spot blotch severity under temperature gradient (with +1, +2, and +3°C above ambient) has been noted. Therefore, direct effect of temperature rise on pathogen and probably indirect effect of changes in host due to CO_2 are likely to contribute a definite increase in spot blotch severity in wheat.

Effect of temperature rise on spot blotch favorable period and developmental rate has been used for forecasting the future spot blotch scenario under climate change regime. Spatial pattern of spot blotch severity throughout Indo-Gangetic plains could be explained based on favorable period and hourly rate of incubation period completion under ambient conditions. Comparatively higher values of favorable hours and rate of incubation period completion in most locations of eastern plains has corresponded well with higher disease severity noted in eastern plains in comparison to western plains. It is also noted that in the hotspots of eastern plains infection may start even early indicating availability of longer favorable period for the region. Under climate change scenario, temperature rise in North Indian plains has been predicted in the range 0.5-2.0°C and rainfall pattern to increase about 20% by the end of century (Kothawale *et al.,* 2010; IPCC, 2013). Adding 0.5-3.0°C over current ambient temperature, prediction of higher level of severity has been reflected. In the present study, although moisture criteria has not been considered in the assessment of temperature impact, but based on increased rainfall forecast in future for the region, it is quite logical to believe higher level of spot blotch severity as moisture level is likely to be increased than the current level. Global forecast on temperature is not uniform as land surface temperature rise was shown to have regions with lower than normal temperature also (Watson, 2001). However, for prediction of developmental rate, the model is robust enough to capture effects of either increase or decrease in temperature.

Simulation of spot blotch favorable period and developmental rates due to elevated temperature has indicated February might be the most critical month when both rates and favorable hours has shown to increase as compared to March. Our

analysis has indicated that favorable hours in February is likely to get increased remarkably (based on % increase) due to addition of 1.5°C over the ambient temperature as minimum temperature gets increased. However, in March, addition of 1.5°C has shown only marginal increase in favorable hours and rates although seasonal temperature goes up. Climate change due to increased level of CO_2 directly known to increase photosynthetic rate and changes on morphological features that might alter crop microclimate in favor of pathogen infection (Chakraborty and Newton, 2011). On the other hand, increased temperatures affect reproductive processes more than they affect photosynthesis and vegetative growth. However, measurable difference or changes in host to favor or disfavor pathogen infection directly or indirectly is an intricate issue. Through elevated CO_2 and temperature (+1.5°C) exposure in growth chamber we have noted increase in leaf area in wheat irrespective of temperature effect and increase in spot size and infected area/cm^2/leaf as well as decrease in incubation and latent period (unpublished). Therefore, direct effect of temperature rise on pathogen and host and probably indirect effect of changes in host are contributing to a definite increase in spot blotch development. Observation on increased spot blotch severity recorded during six seasons in South Asia on a set of local commercial cultivars along with increase in night temperature has been an apprehension in wheat productivity of region (Sharma *et al.*, 2007). In Indian plains, temperature has been continuously shown to increase as the region has already experienced 0.5-0.8°C temperature rise above pre-industrial era (Rupa-Kumar *et al.*, 2006; Kothawale *et al.*, 2010). Recent survey has indicated that spot blotch has gone to non-conventional areas like Pakistan (Shamim *et al.*, 2010) might be the cause of temperature rise. Therefore, current rising trend in spot blotch occurrence reasonably could be ascribed due to rise in temperature.

Overall, the current finding establishes for the first time a criteria for prediction of spot blotch infection for development of a framework for developing another simpler, rules-based forecaster or as a base model in developing a management advisory programme for the stakeholder. The infection criteria or rule would help in the evaluation of breeding materials as the resistant breeding programme against the disease has to be strengthened in the light of expected increase of spot blotch under climate change scenario. Incubation period model in terms of generation or developmental rate could be used for estimation of probable time of spot development rate to optimize fungicidal application as the effective

fungicides are available. Temperature rise is likely to escalate spot blotch infection throughout Indo-Gangetic plains as disease severity is likely to increase further due to availability of longer favorable hours as well as higher rate of incubation period completion.

CHAPTER-IX SUMMARY AND CONCLUSION

Spot blotch in wheat is a fast emerging disease in warmer areas of the world particularly throughout Indo-Gangetic plains. Wheat production is already under terminal heat stress due to rise in minimum temperature under climate change scenario. Spot blotch disease has been proved to be an additional threat as it has become a major production constraint in intensive rice-wheat cropping systems of Indo-Gangetic plains where around 12 million hectare wheat area is affected every year and with an estimated yield loss in the tune of 20-80%. The disease is increasingly becoming a cause of concern particularly in the warm and humid environments of Indian sub-continent. Spot blotch infection is reported to be highly influenced by weather factors mainly temperature and high relative humidity. Forecasting of potential distribution of the disease, as a urgent need, has been done for the assessment of its impact under climate change scenario.

Present study establishes a criterion for spot blotch infection and developmental rate model have been worked out to explain spot blotch development in wheat under climate change scenario. Number of spot /cm^2 leaf (infection index) has been measured in response to the main weather factors namely temperature and duration of relative humidity 95% or above (RH-duration) controlling the development of the pathogen. Based on unimodal temperature response and non-linear monotonic RH-duration response on spot blotch infection a temperature 18-34°C with minimum 15 *h* of high RH found to be essential and defined as favorable period. Temperature response on incubation period has been estimated and incubation period model (IP_{20}) for development of 20% spots from the start of infection. IP_{20} was decreased from 7 days at 18°C to 2 days at 29°C and afterward increased with the increase in temperature. Hourly rate of IP_{20} completion or developmental rate was best described as a linear increase in rate with increase of temperature up to approximately 29°C, then an exponential decline up to the maximum temperature of approximately 36°C in which disease development stopped. A model for hourly IP_{20} completion rate established as $(0.002\ Temp - 0.03)\{1 - exp[0.151(Temp - 36)]\}$ could reasonably explain the rate of spot development for diurnal temperature fluctuations tested under natural infection in the field.

Elevated CO_2 (550 ppm) exposure irrespective of temperature level in growth chamber has shown to increase in leaf area as well as in spot size in wheat but overall

effect as compared to elevated temperature on disease epidemic parameters appeared to be minimum or negligible. However, elevated temperature has appeared to significant in terms of increase in spot size, infected area/cm^2/leaf and decrease in incubation and latent period. Direct effect of temperature rise on the pathogen growth rate and host and probably indirect effect of changes in host due to CO_2 are contributing to spot blotch development as the disease continuously increasing.

Impact of temperature rise on spot blotch favorable hours and developmental rate (IP_{20} completion rate) under current situation has indicated that eastern region of Indo-Gangetic plains is relatively more favorable during February-March than western region. Addition of temperature (1.5°C) above the ambient temperature has shown to increase favorable hours and rate of incubation period completion during February and March, however, proportion of increase in February was more than in March and western plains were shown to be more sensitive to temperature rise.

Current finding establishes the fact

- Occurrence of infection favorable temperature (18-34°C) and RH-duration (15 *h* or above) in crop canopy facilitates spot blotch infection and once infection takes place subsequent progress of the disease is largely dependent on temperature.
- Temperature response on incubation period could be used for prediction of first symptom appearance.
- Temperature rise is likely to escalate spot blotch infection throughout Indo-Gangetic plains as disease severity is likely to increase further due to availability of longer favorable hours as well as higher rate of incubation period completion.

A criterion for monitoring of infection favorable period and a developmental rate has been proposed to develop a spot blotch forecasting system for scheduling protective fungicide applications as the severity of the disease is likely to increase further under climate change scenario.

Forecasting potential distribution of spot blotch in wheat under climate change scenario in Indo-Gangetic plains

Spot blotch (*Bipolaris sorokiniana*) in wheat is one of the most important diseases in warmer areas of the world particularly in South Asia. It has become a major production constraint in intensive rice-wheat cropping systems of Indo-Gangetic plains where around 12 million hectare wheat area is affected every year and yield losses in the tune of 20% to 80% were reported due to the disease. Spot blotch infection is reported to be highly influenced by weather factors mainly temperature and high relative humidity. However, little is known about the importance of small changes in temperature under field conditions either due to seasonal or global warming. The disease is increasingly becoming a cause of concern particularly in the warm and humid environments of Indian sub-continent. Forecasting of potential distribution under climate change requires a prior knowledge of the pathogen's response to the environmental conditions.

In the present study, criteria for spot blotch infection and developmental rate model have been worked out to explain spot blotch development in wheat under climate change scenario. Number of spot /cm^2 leaf (infection index) has been measured in response to the main weather factors namely temperature and duration of relative humidity 95% or above (RH-duration) controlling the development of the pathogen. Temperature response was typically unimodal with cardinal temperatures, i.e. minimum, optimum and maximum, had been appropriated at 18, 29 and 34^oC, respectively. Response of RH-duration was non-linear, which was characterized by a monotonic increase in infection index with the increase of RH-duration. For infection a temperature of 18-34°C with minimum 15 *h* of high RH was found to be required and defined as favorable period. Temperature response on incubation period has been estimated and incubation period model (IP_{20}) for development of 20% spots from the start of infection. IP_{20} was decreased from 7 days at 18°C to 2 days at 29°C and afterward increased with the increase in temperature. Hourly rate of IP_{20} completion was best described as a linear increase in rate with increase of temperature up to approximately 29°C, then an exponential decline up to the maximum temperature of approximately 36°C in which disease development stopped. A model for hourly IP_{20}

completion rate established as *(0.002 Temp –0.03) {1 – exp [0.151(Temp – 36)]}* could reasonably explain the rate of spot development for diurnal temperature fluctuations tested under natural infection in the field.

Elevated CO_2 (450-550 ppm) exposure irrespective of temperature level in growth chamber has shown to increase in leaf area in wheat. Increase in spot size, infected area/cm^2/leaf and decrease in incubation and latent period were noted under combined elevated CO^2 and temperature (+1.5°C) level. Direct effect of temperature rise on pathogen growth rate and host and probably indirect effect of changes in host are contributing to increase in spot blotch development.

Impact of temperature rise on spot blotch favorable hours and rate for IP_{20} completion under current situation has indicated that eastern region of Indo-Gangetic plains is relatively more favorable during February-March than western region. Addition of temperature (1.5°C) above the ambient temperature has shown to increase favorable hours and rate of incubation period completion during February and March, however, proportion of increase in February was more than in March and western plains were shown to be more sensitive to temperature rise.

Current finding establishes the fact

- Occurrence of infection favorable temperature (18-34°C) and RH-duration (15 *h* or above) in crop canopy facilitates spot blotch infection and once infection takes place subsequent progress of the disease is largely dependent on temperature.
- Temperature response on incubation period could be used for prediction of first symptom appearance.
- Temperature rise is likely to escalate spot blotch infection throughout Indo-Gangetic plains as disease severity is likely to increase further due to availability of longer favorable hours as well as higher rate of incubation period completion.

A criterion for monitoring of infection favorable period and a developmental rate has been proposed to develop a spot blotch forecasting system for scheduling protective fungicide applications as the severity of the disease is likely to increase further under climate change scenario.

जलवायु परिवर्तन के परिप्रेक्ष्य के अंतर्गत सिंधु गंगा मैदानी क्षेत्रों मे गेंहू में लगने वाले स्पॉट ब्लॉच रोग के संभावित वितरण का पूर्वानुमान

सार

विश्व के गरम क्षेत्रों , विशेष रूप से दक्षिण एशिया मे गेहूँ का स्पॉट ब्लॉच (बाईपोलेरिस सोरोकीनियाना) सर्वाधिक महत्वपूर्ण रोगो में से एक है। सिंधु गंगा मैदानी क्षेत्रों के सघन धान गेहूं फसल तंत्रो में यह फ़सलोत्पादन में एक प्रमुख अवरोध बन गया जहाँ प्रतिवर्ष लगभग १२ मिलियन हेक्टयर गेहूं क्षेत्र इस रोग से प्रभावित होता है और इस रोग के कारण २० % से ८० % तक क्षति सूचित की गई है । मौसम करको मुख्यतया तापमान एवं उच्च आपेक्षिक आद्रता द्वारा स्पॉट ब्लॉच संक्रमण पर अत्यधिक प्रभाव सूचित किया गया है। वैसे प्रक्षेत्र परिस्थितियों के अंतर्गत तापमान में ऋतु संबंधी या वैश्विक तापमान के कारण होने वाले थोड़े थोड़े परिवर्तनों के महत्व के विषय में बहुत कम जानकारी उपलब्ध है। यह रोग विशेष रूप से भारतीय उप महाद्वीप के गरम एवं आद्र वातावरणों में चिंता का विषय बनता जा रहा है। जलवायु परिवर्तन के अंतर्गत रोग के संभावित वितरण के पूर्वानुमान हेतु पहले पर्यावरण परिस्थितियों के प्रति रोग जनक की अनुक्रिया के बारें मे जानना आवश्यक है । प्रस्तुत अध्यन में, जलवायु परिवर्तन के परिप्रेक्ष्य में स्पॉट ब्लॉच रोग के विकास की व्याख्या हेतु, स्पॉट ब्लॉच के संक्रमणार्थ एक मापदंड एवं विकास दर संबंधी मॉडल तैयार किया गया है। रोगजनक के विकास को नियंत्रित करने वाले मुख्य मौसम करको नामतः तापमान एवं ९५ % अथवा अधिक आपेक्षिक आद्रता की अवधि (आर एच अवधि) की अनुक्रिया में प्रतिवर्ग सेमी पर्ण क्षेत्र पर धब्बों की संख्या (संक्रमण घातांक) ज्ञात किए गए। तापमान अनुक्रिया मुख्य तापमानों अर्थात

न्यूनतम ईष्टतम एवं अधिकतम के साथ आदर्श रुपेण एकबहुलकी थी जिन्हे क्रमशः १८, २९ एवं ३४° से निर्धारित किया गया। आर एच अवधि की अनुक्रिया आरेखीय थी जिसका आर एच अवधि बढ़ने के साथ , संक्रमण घातांक में एकदिष्ट बढ़ोत्तरी के द्वारा अभिलक्षण किया गया। संक्रमण हेतु न्यूनतम १५ घंटे की उच्च आर एच के साथ १८- ३४° से तापमान का बने रहना आवश्यक पाया गया और इसे अनुकूल अवधि के रूप में परिभाषित किया गया। उदभवन अवधि पर तापमान अनुक्रिया का आकलन किया गया और संक्रमण की शुरुआत से २० % धब्बों के विकास हेतु उदभवन अवधि मॉडल (आई पी २०) तैयार किया गया। आई पी २० , १८° से तापमान पर ७ दिनों से लेकर २९° से पर २ दिन तक कम हुआ तथा उसके बाद तापमान बड़ोत्तरी के साथ बढ़ गया। तापमान में लगभग २९ ° तक बढ़ोत्तरी के साथ , आई पी २० समापन की प्रतिघण्टा दर को , दर में रेखीय बढ़ोत्तरी के रूप में भली भांति वर्णित किया जा सका, तत्पश्चात तापमान में लगभग ३६ ° की अधिकतम बढ़ोत्तरी होने पर चरघातांकी कमी देखी गई जिस पर रोग विकास रुक गया। घंटा दर घंटा आई पी २० समापन दर हेतु, (०.००२ तापमान – ०.०३) [१- exp{ ०.१५१(तापमान – ३६)}] के रूप में स्थापित एक मॉडल , खेत में प्राकर्तिक संक्रमण के अंतर्गत परीक्षण किए गए दैनिक तापमान- परिवर्तनों हेतु धब्बों की विकास दर की स्पष्ट रूप से व्याख्या कर सका। व्रद्धि कक्ष में गेहूं के पोधों को प्रवर्धित CO_2 (४५०-५०० पीपीएम) में रखने से, तापमान से अप्रभावित पर्ण क्षेत्रफल में व्रद्धि देखी गई है। प्रवर्धित CO_2 एवं तापमान (+ १.५°) स्तर के सयुंक्त प्रभाव के अंतर्गत क्षत- परिमाण एवं संक्रमित क्षेत्र/ सेमी ^{2} /पत्ती में बढ़ोत्तरी तथा उदभवन एवं गुप्त अवधि में कमी देखी गई। आतिक्षेय एवं रोगजनक की व्रद्धि दर पर तापमान में बढ़ोत्तरी का प्रत्यक्ष प्रभाव तथा आतिक्षेय में परिवर्तनों के परोक्ष प्रभाव , संभवतया स्पॉट ब्लॉच रोग के विकास में योगदान करते हैं। वर्तमान परिस्थितियो में , तापमान मे बढ़ोत्तरी का स्पॉट ब्लोच के लिए अनुकूल

घंटो तथा आई पी २० समापन हेतु दर पर तापमान में बढ़ोत्तरी को दर्शाता है कि फरवरी एवं मार्च के दौरान सिंधु – गंगा मैदानो के पश्चिमी क्षेत्रों कि तुलना में पूर्वी क्षेत्र अपेक्षाकृत अधिक अनुकूल है। फरवरी एवं मार्च के दौरान परिवेशी तापमान के ऊपर तापमान (१.५°) बढ़ोत्तरी, अनुकूल घंटो एवं उदभवन अवधि समापन कि दर में बढ़ोत्तरी को दर्शाता है , वैसे मार्च कि तुलना में फरवरी में बढ़ोत्तरी का अनुपात अधिक था तथा तापमान में बढ़ोत्तरी के प्रति पश्चिमी मैदानी क्षेत्र अधिक संवेदनशील पाए गए ।

इन अध्यनों से निष्कर्ष निकलता है कि फसल वितान में संक्रमण के अनुकूल तापमान (१८- ३४°) एवं आर एच अवधि (१५ घंटे अथवा अधिक) का बने रहना, स्पॉट ब्लॉच संक्रमण में सहायक औए जब एक बार सक्रमण हो जाता है रोग का आगे फैलना प्रमुखताया तापमान पर निर्भर करता है। प्रथम रोग लक्षणों के प्रकट होने कि भविष्यवाणी हेतु उदभवन अवधि पर तापमान अनुक्रिया का उपयोग किया जा सकता है। तापमान में बढ़ोत्तरी होने से संपूर्णा सिंधु गंगा मैदानी क्षेत्र में स्पॉट ब्लॉच संक्रमण तेजी से बढ़ने कि संभावना है क्योकि अधिक अनुकूल घंटो के साथ साथ उदभवन अवधि समापन कि उच्चतर दर उपलब्ध होने से रोग उग्रता के और अधिक बढ़ने कि संभावना है। सुरक्षात्मक कवकनाशी अनुप्रयोगो हेतु एक स्पॉट ब्लॉच पूर्वानुमान तंत्र विकसित करने के लिए संक्रमणार्थ अनुकूल अवधि एवं विकास संबंधी दर की देख रेख हेतु एक उपाय का सुझाव दिया गया है क्योंकि जलवायु परिवर्तन के बदलते परिप्रेक्ष्य में इस रोग कि उग्रता कि संभावना है ।

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