

# Forewarning models for *Sclerotinia* rot (*Sclerotinia sclerotiorum*) in Indian mustard (*Brassica juncea* L.)

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**Abstract** *Sclerotinia sclerotiorum* (Lib.) de Bary has worldwide distribution and causes diseases in more than 500 host plants. *Sclerotinia* rot is a menace to cultivation of oilseed *Brassica* crops worldwide. The epidemiology of *Sclerotinia* rot (SR) of Indian mustard (*Brassica juncea* L.) was investigated during 2004–2012 crop seasons, and based on 8 year of disease data. The forecasting models were developed first time in Indian conditions and then validated in 2012–13. The carpogenic infection initiated in 52 standard week (last week of December) and continued during 1 to 3 standard weeks (first three weeks of January). Disease first appeared after closure of the crop canopy when flowering started. During epidemics, the 8 year mean daily maximum and minimum air temperature was 19.4 and 5.1°C, morning and afternoon RH 95 and 62 per cent, bright sunshine hours 4.9 and rainfall was 1.4 mm, all are conditions favourable for disease development. The  $R^2$  value of the regression analysis between observed and estimated SR prevalence was 0.98. Disease forecasting could provide the growers with information for well timed application of fungicides to control SR and this would be beneficial economically.

**Key words** *Sclerotinia sclerotiorum* · disease forecasting · ascospores · sclerotia · Indian mustard

## Introduction

*Sclerotinia* rot (SR) caused by *Sclerotinia sclerotiorum* (Lib) de Bary, is a ubiquitous, omnivorous, soil-borne and destructive plant pathogen distributed worldwide. The pathogen is known to infect more than 500 plant species of diverse phylogenetic backgrounds including 278 genera, 75 families of dicotyledonous and a number of significant monocotyledonous plants (Boland and Hall, 1994; Saharan and Mehta, 2008). In India, *Sclerotinia* rot is a menace to cultivation of Indian mustard [*Brassica juncea* (L.) Czern & Coss] where the crop is mainly grown in the states of Rajasthan, Haryana, Madhya Pradesh, Uttar Pradesh, Gujarat, West Bengal and Bihar (Aggarwal *et al.* 1997). In fact, the disease incidence has been reported up to 80% in Punjab and Haryana (Kang and Chahal, 2000; Sharma *et al.* 2001). In Rajasthan, about 40% seed yield loss was observed in 60% disease incidence during maturity period of the crop (Chattopadhyay *et al.* 2003).

It has been reported that continuous moisture for about 10 days is required for apothecial development and even a slight moisture tension prevents apothecial formation. Approximately 48–72 hrs of continuous leaf wetness is required for infection by ascospores. Bom and Boland (2000) evaluated environmental variables for forecasting SR of canola and indicated that soil moisture was most closely associated with the

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development of disease when petal infestation was high. Petal infestation and soil moisture levels are the best factors to use in a predictive model of *B. juncea*. Sclerotinia rot was positively correlated with increase in soil moisture and relative humidity at flowering period (Sharma *et al.* 2009).

Ascospore germination, growth and lesion initiation and development are optimal factors needed to develop for crop epidemics (Abawi and Grogan, 1975; Singh and Tripathi, 2001). Air borne ascospores and soil borne hyphae are important primary inocula for epidemics of Sclerotinia rot. Sharma *et al.* (2010) observed petal infection with ascospores during full bloom stage and found rainfall to be an important factor in carpogenic infection of *S. sclerotiorum* in *B. juncea*. Ascospores do not germinate when directly lands on leaf surface, but if the ascospores lands on petals, then germinate and infect the petal tissue. Infected petals in contact with leaves are essential to the development of SR (Jamaux and Spire, 1994). Mwiindilila and Hall (1990) reported that the incidence of white mold was significantly correlated with the canopy coverage of bean during the entire bloom period. The amount and distribution of rainfall during this period were also critical for the development of a canopy favorable to the disease. Hall and Mwiindilila (2000) further provided quantitative information on the duration and magnitude of factors, such as flowering, canopy density, moisture, and the presence of apothecia within the field, in relation to disease development.

A forecast of SR based on petal infestation during early bloom were relatively accurate when disease risk and incidence were low, but they were less reliable when disease risk and incidence were moderate to high (Turkington *et al.*, 1991a). Although the overall success rate was high, factors such as rainfall and density of crop canopy, which affect ascospore production and release, were found to influence the relationship between petal infestation and disease incidence (Turkington *et al.*, 1991a; Hall and Mwiindilila, 2000).

Little information is available on the epidemiology of Sclerotinia rot of Indian mustard under field conditions. Thus, a study was undertaken with the objectives to examine selected environmental, crop and pathogen variables recorded from an Indian mustard (*Brassica juncea*) field, to determine the most effective method of predicting Sclerotinia rot disease.

## Materials and Methods

**Field Sites** A field (19 X 21.6 m) of Indian mustard was established at an experimental farm of the ICAR- Directorate of Rapeseed-Mustard Research (DRMR), Bharatpur, India (77°27 E, 27°12 N; 178.13 m MSL). The plot had a history of Sclerotinia rot of mustard, and having loam type soil of alkaline pH (8.0) with tropical dry semi arid climate. The plot was prepared for planting by post rainy season ploughing and planking. *Brassica juncea* cv. Rohini (Seed Production Unit, DRMR) was seeded on 3 sowing dates (8 and 29 October and 19 November) starting from first week of October with three-week intervals using 4.8X5 m plot size with 4 replications. Seeds were sown with 30x10 cm, row to row and plant to plant spacing respectively. Farmers of Indian mustard growing area sown their crop during first to fourth week of October, hence two seeding dates of October were taken while one late sown seeding date was chosen to observe SR incidence. The SR incidence and weather data were continuously collected from years 2004 to 2012 and validated during 2013 using a suitable model.

**Data Collection** Data for weekly soil moisture, disease incidence were recorded, and weather data were used to identify the suitable conditions for disease development. Data on different weather variables were collected from meteorological observatory established by Indian Meteorological Department (IMD) at experimental farm, DRMR, Bharatpur. Air temperature was monitored with a hygrothermograph in a Stevenson shelter. Rainfall was recorded using a rain gauge. Bright sun shine hours (BSSH) recorded with a Campbell-Stokes recorder and average evaporation per day as measured by the Class A evaporation pan. Soil moisture at weekly interval recorded from each replicated plots with gravimetric method, fresh and oven dry weight were taken and per cent soil moisture calculated. For confirmation of disease initiation in field, petals were sampled weekly from before commencing flowering, and continuing until completion of petal fall. Throughout the flowering period, 20 petal samples from each replication were collected in individual Petri dishes and transported to the laboratory during the early morning hours. Petals were stored at 4°C until culturing, on the same day. Four petals were plated on each Petri plate and replicated thrice for each replicate plot on rose bengal agar, 39 g L potato dextrose agar (Hi media) amended with 30 ppm

rose bengal (Eastman Organic Chemicals, Rochester, NY) and 20 ppm streptomycin sulphate (Sigma Chemical Co., St. Louis, MO). The petals were incubated at  $22\pm 2$  °C for 5 days. A preliminary rating of the number of petals developing *S. sclerotiorum* colonies was made after 5 to 6 days. This preliminary identification of *S. sclerotiorum* colonies was confirmed after 12 days with the presence of sclerotia on the fungal colony. Per cent petal infection (PPI) was calculated by total number of petals infected divided by total number of the plated petals and percentage was calculated.

Disease incidence was determined prior to crop swathing, calculated as the number of diseased plants expressed as a percentage of the total number of plants in each plot.

**Weather Indices (WI) based regression models** Weather indices were obtained and used as predictors for model development. In this type of model, for each weather variable, two indices were developed. One as simple total of values of weather variables in different weeks, and the other one as weighted total, weighted values were being used for determining correlation coefficients between variable to forecast and weather variable in respective weeks. The first index represents the total amount of weather variable received by the crop during the period under consideration while the other one takes care of distribution of weather variable with special reference to its importance in different weeks in relation to the variable to forecast. On similar lines, indices were computed with products of weather variables (taken two at a time) for joint effects (Agrawal *et al.* 1986; Chattopadhyay *et al.* 2005; Desai *et al.* 2004; Dhar *et al.* 2007; Agrawal and Mehta, 2007; Kumar, 2013).

The form of the model was

$$Y = a_0 + \sum_{i=1}^p \sum_{j=0}^1 a_{ij} Z_{ij} + \sum_{i \neq i'}^p \sum_{j=0}^1 b_{ii'j} Z_{ii'j} + \varepsilon$$

where,

$$Z_{ij} = \sum_{w=n_1}^{n_2} r_{iw}^j X_{iw}$$

$$Z_{ii'j} = \sum_{w=n_1}^{n_2} r_{ii'w}^j X_{iw} X_{i'w}$$

Y is variable to forecast

$X_{iw}$  is value of  $i^{\text{th}}$  weather variable in  $w^{\text{th}}$  week

- $r_{iw}$  is correlation coefficient between Y and  $i^{\text{th}}$  weather variable in  $w^{\text{th}}$  week
- $r_{ii'w}$  is correlation coefficient between Y and product of  $X_i$  and  $X_{i'}$  in  $w^{\text{th}}$  week
- p is number of weather variables
- $n_1$  is initial week for which weather data was included in the model
- $n_2$  is final week for which weather data was included in the model, and
- e is error term

Weather variables on maximum and minimum temperature, morning and evening relative humidity, and bright sunshine hours (T max, T min, RH mor, RHeve and BSSH –  $X_1$  to  $X_5$ ) for the period from 2004-05 to 2011-12 were considered for models development. Models have been validated using data on subsequent years (2011-12) which were not included in developing the models. Stepwise regression technique was used for selecting important variables to be included in the model. A forecast performance is defined in terms of the forecasting error, which is the difference between the observed and the predicted value. The forecast performance of models were judged by Mean Absolute Percentage Error (MAPE).

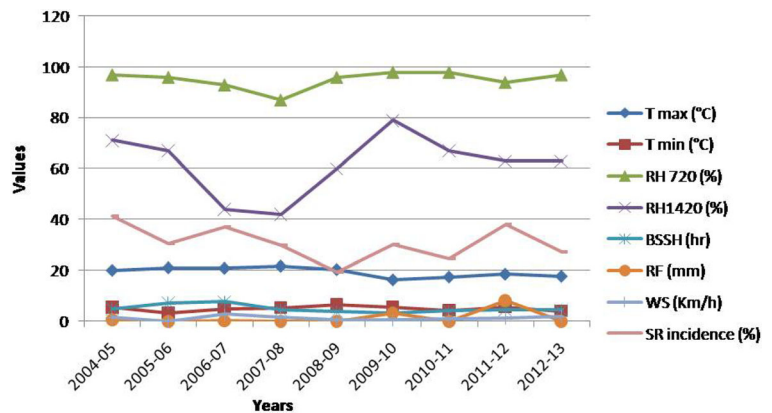
$$MAPE = \frac{1}{n} \sum \left| \frac{(Y_t - \hat{Y}_t)}{Y_t} \right| \times 100$$

where,  $Y_t$  is actual observation,  $\hat{Y}_t$  is the forecast from model and n is the total number of test data point.

## Results

Sclerotinia rot was initially observed in the experimental trial after crop flowering had started. Lesions of Sclerotinia rot were most frequently observed on the stem and petiole junction which may be due to inoculum load available in the field occurs through germination of apothecia released ascospores. Lesions were also observed on leaves where shredded petals were present, which play an important role as sole media for the pathogen development. These lesions expanded to the main stem by direct mycelial colonization of tissues. Ascospore germination and lesion development were similar to earlier studies (Abawi and Grogan, 1975; Singh and Tripathi, 2001). Disease incidence continued

**Fig. 1** Effect of different weather variables on per cent Sclerotinia incidence in *B. juncea* over the years. **Tmax(°C)**: Maximum temperature (°C); **Tmin(°C)**: Minimum temperature (°C); **RH 720(%)**: Morning Relative humidity (%) at 7:20 AM; **RH 1420(%)**: Afternoon Relative humidity (%) at 2:20 PM; **BSSH (hr)**: Bright Sun Shine Hours; **RF (mm)**: Rainfall (mm); **WS (Km/h)**: wind speed (Km/h); **SR incidence (%)**: Sclerotinia rot incidence (%)



to increase until favourable weather conditions and reached 41.2% (2004-05), 36.6% (2005-06), 37.1% (2006-07), 29.8% (2007-08), 19.4% (2008-09), 30.1% (2009-10), 24.6% (2010-11), 37.9% (2011-12), and 27.1% in 2012-13 (Fig. 1). The initial appearance of Sclerotinia rot lesions was associated with low temperature; high relative humidity in the morning and afternoon, low bright sun shine hours and rainfall during this period. The mean of the 3 seeding dates during last 8 years daily mean maximum air temperature was 19.4 °C (17.8-21.7 °C), minimum 5.1 °C (3.4-6.6 °C), morning RH 95 per cent (87-98%), afternoon RH 62 per cent (42-79%), bright sunshine hours 4.9 (3.1-7.7) and rainfall was 1.4 mm (0.0-8.2 mm) (Table-1). The regression models using weather indices were developed viz. 8<sup>th</sup>, 29<sup>th</sup> October and 19<sup>th</sup> November. The model for per cent Sclerotinia rot incidence in different date of planting along with coefficient of determination, and forecast along with observed value during 2011-12 is presented in Table 2. The interaction of minimum temperature and

relative humidity in the evening was most significant variable responsible for development of % Sclerotinia rot incidence in mustard sown on 8 October. Relative humidity in the morning and evening along with the number of bright sunshine hours were most significant variables responsible for disease development in crop sown on 29 October, where maximum SR incidence was observed. On 19 November sown crop, minimum temperature along with the interaction of relative humidity and bright sun shine hours were most significant variable responsible for infestation of % Sclerotinia rot incidence in mustard crop. Predicted and observed value in different dates (8 October, 29 October and 19 November) are presented in Fig. 2 reflected good agreement between two of predicted and observed value of % Sclerotinia rot incidence in different planting dates.

Soil moisture was also recorded since 2008-09 to 2012-13 (5 years) and models were developed (Table 3), which indicated soil moisture as an important variable in SR infestation. Per cent petal infection (PPI)

**Table 1** Mean weather variables of 3 seeding dates during epidemics of Sclerotinia rot (1-3 standard week)

Year	T max (°C)	T min (°C)	RH mor (7:20 AM)	RH eve (14:20 PM)	BSSH (hr)	RF (mm)	SR incidence (%)
2004-05	20.1	5.5	97	71	4.9	0.6	41.2
2005-06	21.1	3.4	96	67	7.2	0.0	30.6
2006-07	20.9	5.0	93	44	7.7	0.2	37.1
2007-08	21.7	5.3	87	42	4.3	0.0	29.8
2008-09	20.4	6.6	96	60	3.8	0.0	19.4
2009-10	16.4	5.7	98	79	3.1	3.3	30.1
2010-11	17.5	4.4	98	67	4.0	0.1	24.6
2011-12	18.6	5.6	94	63	4.5	8.2	37.9
2012-13	17.8	4.0	97	63	4.4	0.0	27.1
Average	19.4	5.1	95.0	62	4.9	1.4	

**Table 2** Epidemiology models for % Sclerotinia rot incidence in different date of planting along with coefficient of determination and forecast and observed value in 2011-12.

Date of planting	Models	R <sup>2</sup>	% Disease forecast 2011-12	
			observed	forecast
8 Oct	$Y=3.93 + 0.002 * Z_{341}$	0.97	28.0	24.3
29 Oct	$Y= 51.296 + 0.958 Z_{50} + 0.289 Z_{31} -0.164 Z_{41}$ (0.93) (0.03) (0.02)	0.98	37.9	32.9
19 Nov	$Y= 8.276 + 1.6217 Z_{21} + 0.013 Z_{451}$ (0.89) (.09)	0.98	18.8	19.6

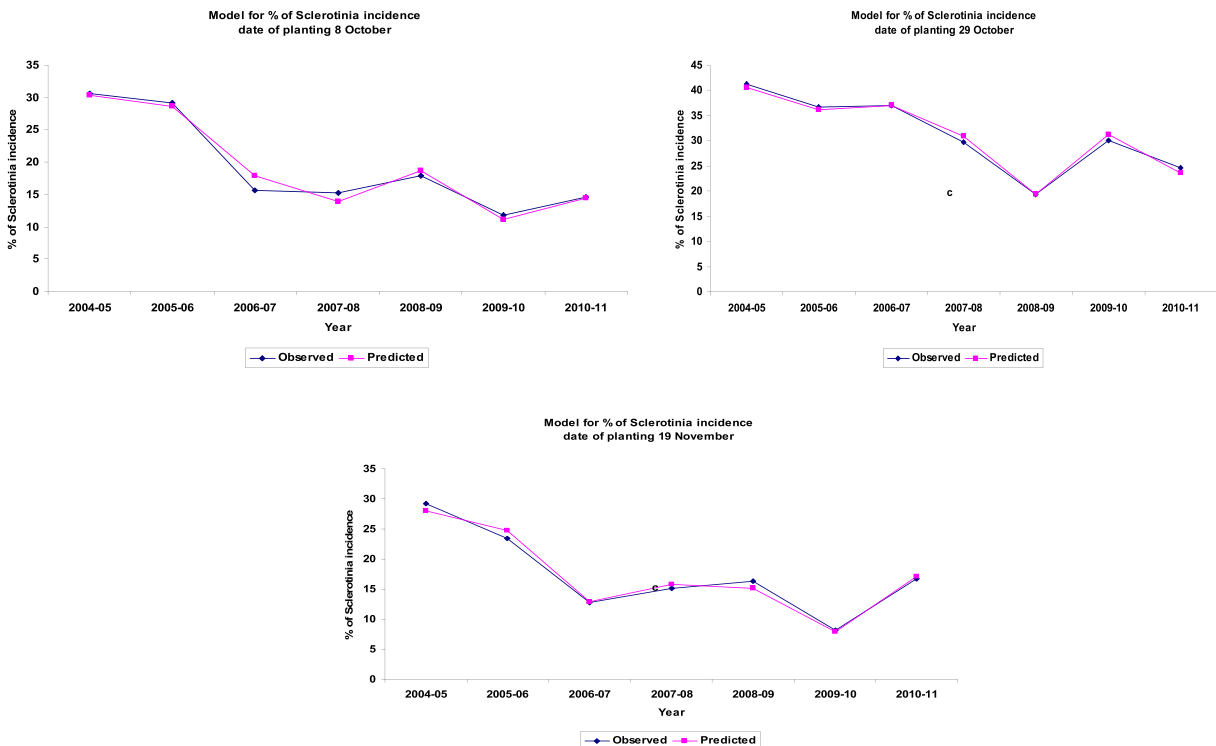
$Z_{341}$  is weighted interaction of relative humidity in morning with relative humidity in evening;  $Z_{50}$  is unweighted subshine-hour;  $Z_{31}$  is weighted relative humidity in morning ;  $Z_{41}$  is weighted relative humidity in evening ;  $Z_{21}$  is weighted minimum temperature and  $Z_{451}$  is weighted interaction of relative humidity in evening with sun shine-hour. In brackets, the contribution of selected variables are reported

resulted, initiation of SR incidence in first standard week in all dates of seeding which was maximum in 29 Oct sown crop. PPI increased in 2 and 3 standard week in 8 and 29 Oct sown crop (Table 4).

## Discussion

An effective disease prediction model for Sclerotinia rot in Indian mustard must account for the amount of

inoculum present, the susceptible stages of crop development, and the suitability of environmental conditions for initiation of disease. In various crops, petal infestation (Gugel and Morrall, 1986) and apothecial counts (Boland and Hall, 1988a,b) have been proposed as measures of available inoculum of *S. sclerotiorum*, and rainfall and soil moisture have been used to assess environmental conditions. Results from this study establish that measurement of weather variables, and soil moisture can improve the ability to identify mustard

**Fig. 2** Predicted and observed value of per cent Sclerotinia incidence in different date (8 October, 29 October and 19 November)

**Table 3** Models developed using data from 2008-09 to 2012-13 (Tmax Tmin RHmor, RHeve, BSSH and soil moisture).

Date of planting	Models	R <sup>2</sup>	% Disease forecast 2011-12	
			observed	forecast
8 October	Y= 4.550 + 0.017 * Z <sub>161</sub>	0.81	17.2	17.1
29 October	Y= 21.585 + 0.023 * Z <sub>161</sub>	0.77	27.8	27.7
19 November	Y = 4.270 + 0.014 * Z <sub>161</sub>	0.66	13.9	13.8

Z<sub>161</sub> is weighted interaction of maximum temperature and soil moisture

crops that are at risk of economically important outbreaks of Sclerotinia rot in oilseed Brassica growing areas. Quantification of the amount of petal infestation by determining the percentage of petals that develop colonies of *S. sclerotiorum* on rose bengal agar can provide a useful measurement of inoculum. This method takes into account both externally and internally produced inoculum, and low levels of inoculum are probably more easily detected by petal plating than by counting apothecia. The conditions under which petals are collected can influence the percentage of petal infestation measured. For example, a mean increase of 34% petal infestation was observed in samples collected from morning to mid-afternoon. When wet petals were collected or collected prior to a heavy rain, disease could be underestimated (Turkington, 1988). Additional factors such as sample size, canopy density, changing inoculum levels during crop flowering, diurnal and weather-related inoculum fluctuations, rainfall, light penetration, leaf area index and crop height can affect the relationship between petal infestation and incidence of Sclerotinia rot (Turkington *et al.* 1988; 1991a,b; Turkington and Morrall, 1993).

Environmental conditions are another important factor in the development of disease. In this study, relative humidity, BSSH and temperature were the variables

**Table 4** Per cent petal infection observed during 2008-09 to 2012-13.

Standard week	Per cent Petal Infection (PPI)*		
	8 Oct	29 Oct	19 Nov
1	9.4	11.0	2.0
2	19.9	21.9	3.8
3	16.1	18.4	2.0

\*Mean of 5 years (2008-09 to 2012-13)

most closely associated with disease development when petal infestation was high. During the petal infestation, soil moisture was also recorded maximum. There are several possible explanations for the importance of soil moisture in prediction of disease caused by *S. sclerotiorum*. Soil moisture values may also be an indication of the general availability of moisture within the canopy. If soil moisture is high, relative humidity levels within the canopy will be higher than those above the crop canopy. If soil moisture is low, however, there will not be enough available moisture to increase the relative humidity within the canopy for infection to develop. Turkington (1988) suggested that a dense canopy would decrease evaporation and, thereby prolong the period of favourable moisture conditions within the canopy. Hunter *et al.* (1984) suggested that a measure of soil moisture is an indication of sclerotial germination and, thereby, of ascospore production, rather than of disease development. However, soil moisture within a crop may also reflect environment conditions associated with rain or dew that are suitable for disease development. According to the data available in this study, several combinations of relative humidity, maximum and minimum temperature and BSSH could be used for disease prediction. Turkington (1988) found that petal sampling during the early stages of flowering generally gave better results than later sampling, although this relationship was variable (Turkington and Morrall, 1993). There is need to develop statistically sound objective forecasts of crop yield on the basis of weather variables so that reliable forecasts can be obtained. Thus extent of weather influence on pests infestation depends not only on the magnitude of weather variables but also on the distribution pattern of weather over the crop season which, as such, calls for the necessity of dividing the whole crop season into fine intervals and studying the relationship in these periods. This will increase number of variables

in the model and in turn a large number of model parameters will have to be evaluated from the data. This will require a long series of data for precise estimation of the parameters which may not be available in practice. Thus, a technique based on relatively smaller number of manageable parameters and at the same time taking care of entire weather distribution may solve the problem. Therefore, for each weather variable two indices were developed, one as simple total of values of weather variables in different weeks and the other one as weighted total, weights being correlation coefficients between diseases infestation and weather variable in respective weeks. The first index represents the total amount of weather variable received by the crop during the period under consideration while the other one takes care of distribution of weather variable with special reference to its importance in different weeks in relation to the infestation of diseases. On similar lines, indices were computed with products of weather variables (taken two at a time) for joint effects. Stepwise regression technique was used for selecting important variables to be included in the model. The forecasts have been obtained for subsequent years not included in model development. The performances of the forecasts were judged with mean absolute percentage error (MAPE).

During the study, models used the weighted index (distribution of weather variables to crop / diseases) and unweighted index (total accumulation of weather received by crop /diseases) for development of models. Therefore, the index distribution of the weather variable or products of the weather variables used to study, the combined effects of weather on disease which are necessary for models development. Weather and soil moisture data were used since 41 to 52 standard week so that SR prediction can give to farmers well in advance. However, PPI initiated in first standard week, hence PPI was not included in the models. Turkington (1988) also noted that petal sampling must be done when inoculum was present and not at any particular growth stage. Therefore, sampling should be conducted throughout the flowering period. The fact that inoculum levels and environmental suitability must coincide lends itself to the use of a stepwise sampling technique for the prediction of disease. Further, the study was conducted on the effectiveness of fungicide applications with respect to prediction model and which was found very accurate (Mwiindilila and Hall, 1989).

This study clearly indicates that favourable environmental conditions must coincide with sufficient

inoculum before Sclerotinia rot will be a problem in Indian mustard. Soil moisture levels, BSSH and temperature appear to be the best factors to use in a predictive model. Relationship among minimum temperature, relative humidity, and sun shine hours as well as soil moisture required for carpogenic germination derived in this study, provide a basis for predication. However, the field data showed that there is still some other outstanding effects of weather variables to be understood before an accurate prediction of SR can be achieved. Monitoring of disease should start by growers when petals start to fall on the soil. These results indicated the need to develop disease prediction system for improving the timing of application and efficacy of control measures, to minimize the threat of SR.

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