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Development of Leaf Spot (*Myrothecium roridum*) and Dispersal of Inoculum in Mulberry (*Morus* spp.)

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Studies were conducted on the effect of pruning time, host age, conidial dispersal and weather parameters on the incidence and severity of mulberry leaf spot (*Myrothecium roridum*). The disease severity (%) increased with increase in shoot age irrespective of pruning date. Maximum disease severity was observed in plants pruned during first week of April and minimum disease severity in plants pruned during first week of March. Significant ($P < 0.01$) influence of date of pruning, shoot age and their interaction was observed on severity of the disease. Apparent infection rate (r) was significantly higher during the plant growth period from day 48 to day 55. Average apparent rate was higher in plants pruned during first week of April and least in plants pruned during first week of July. The disease infection was negatively correlated to distance from the inoculum source. Leaf spot severity (%) was influenced by weather parameters. Multiple regression analysis revealed contribution of various combinations of weather parameters on the disease severity. Linear prediction model ($Y = -81.803 + 1.176x_2 + 0.765x_3$) with significant R^2 was developed for prediction of the disease under natural epiphytotic condition.

Key words: Mulberry, *Myrothecium roridum*, Disease severity, Shoot age, Pruning date, Apparent infection rate, Inoculum dispersal

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Introduction

Mulberry (*Morus* spp.) is the only food plant of silkworm (*Bombyx mori* L.). Leaf spot caused by *Myrothecium roridum* is an economically important disease of mulberry, which reduce quantity and quality of silk produced (Qadri *et al.*, 1999). The disease cause deterioration in nutritive value of mulberry leaves by changing biochemical constituents and altering physiological characters of the leaves (Shree and Nataraja, 1993; Pratheesh Kumar, *et al.*, 2002). Though some chemicals are reported for the control of this disease (Govindaiah *et al.*, 1988), management using chemical fungicides is not economical, besides many chemicals are reported toxic to silkworms. In addition, they are non-degradable and non eco-friendly. Disease escape is one of an important aspect for protection of crop plants from disease attack for a better harvest. Since the disease is an interaction of susceptible host, virulent pathogen and favorable weather, age of the plant at a particular weather condition that favours the pathogen is important for its development. Studies on host age and disease development were done in many agricultural crops (Furgo *et al.*, 1994; Srivastava and Khan, 1994; Sekhon and Sokhi, 1999) and in mulberry (Pratheesh Kumar *et al.*, 2000). However, possibility of cultural management of *Myrothecium* leaf spot in mulberry is yet to be exploited. This study was conducted to evaluate different dates of pruning on the severity and development of the disease, influence of weather factors and dissemination of inoculum.

Materials and Methods

The experiment was conducted at the research farm of Central Sericultural Research & Training Institute, Berhampore, West Bengal (tropical humid, latitude-24°6' N, latitude-88°15' E, and altitude 19 m above mean sea level). The experiment was conducted during 1998 and 1999. A popular mulberry variety S1 was used in this study.

Mulberry plants were raised following standard cultural practices (Subba Rao, 1989). Plants with shoot age difference of 7-days were obtained by weekly rotational pruning. Leaf spot severity on 48-, 55-, 62-, 69-, 76-, 83-, and 90-day-old shoots were recorded from plants pruned during first second, third and fourth week of all the months. Ninety-day-old plants were finally pruned off to repeat the process. Observations on the leaf spot severity were recorded using a scale of 0 – 5 (Govindaiah *et al.*, 1989). This rotational pruning was made for two consecutive years. Data on the epidemiological factors such as temperature (°C), relative humidity (%) and rainfall (mm) were recorded daily. Disease severity (%) was calculated using standard formula (Govindaiah *et al.*, 1989).

Apparent infection rate (r) per unit per day was calculated using formula of van der Plank (1963), as follows:

$$r = \frac{2.303}{t_2 - t_1} \log_{10} \frac{X_2(1-X_1)}{X_1(1-X_2)}$$

where,
r = rate of disease development
t_1 = date of first observation
t_2 = date of second observation
X_1 = disease severity in first observation
X_2 = disease severity in second observation.

Since weather condition from June to November favour occurrence and development of the disease, severity and apparent infection rate on shoots of various age groups (plants pruned during different weeks of March to August) during this period was only considered for establishment of influence of plant growth period and pruning dates on severity and apparent infection rate. These data for two years were pooled and evaluated for two factors ANOVA and means were compared for significant difference.

Data on severity of disease and meteorological parameters were pooled for two years and multiple regression equation were derived using the meteorological factors such as mean maximum (x_1) and minimum (x_2) temperatures, per cent relative maximum (x_3), minimum (x_4) humidity, and rainfall (x_5) recorded daily from meteorological observatory near the experimental site. The predicted mean disease severity index (Y) equation, *i.e.*, $Y= a_1 + b_1x_1 + b_2x_2 + \ldots + b_5x_5$ was derived by multiple regression analysis. Where Y denotes the predicted disease severity, a denotes the intercept and b_1 to b_5 denote regression coefficient for x_1 to x_5 weather factors. Significance of coefficient of multiple determination (R^2) and partial regression coefficient (b) values were followed at $P < 0.05$ (Snedecor and Cochran, 1967).

Dissemination of inoculum was studied in the rainy season (June - July) during the year 1999, using one-year-old mulberry plants raised in earthen pots (8" inner dia. x 12" ht.) following recommended package of practices. These plants were pruned and after 30 days these plants were inoculated with conidial suspension (10^6 /ml) of the fungi and allowed the disease to develop in the ambient weather condition were used as the inoculum source. After the diseases developed to > 50% on these plants, the trap plants that are not having any pathogen infection were placed at 20, 40, 60, 80, 100 cm away from the source plants. These whole sets were then exposed to rain. Three such sets were exposed to three different intensities *i.e.*, 20.8 mm, 28.5 mm, and 34.2 mm of rain. Immediately after exposure to rain, the trap plants were removed and incubated at laboratory condition. The number of spots developed on trap plants placed at different distances was monitored for 14 days. Correlation analysis was done on the number of spots and distance from the source of inoculum.

Results

Myrothecium leaf spot severity was higher in plants pruned during first, second and third week of April (Table 1). Analysis of variance revealed significant influence of date of pruning ($F = 38.54$, $P < 0.01$), shoot age ($F = 161.76$, $P < 0.01$) and date of pruning × shoot age interaction ($F = 8.86$, $P < 0.01$) on *Myrothecium* leaf spot severity. Severity was significantly ($P < 0.01$) increased with increasing shoot age, irrespective of pruning time with average maximum disease severity (6.42%) in 90-day-old shoots and minimum (1.10%) in 48-day-old shoots. Maximum disease severity (18.87%) was noticed in 90-day-old shoots, which were pruned during first week of April. Plants pruned before fourth week of March showed significantly less disease severity irrespective of shoot age. Average disease severity (7.56%) was observed in plants pruned during the first week of April and the least in plants pruned during first week of March (0.12%). However, mean disease severity was not significantly differed among plants pruned during first, second and third week of March, plants pruned from May first week to June second week and among plants pruned after third week of July.

Infection rate (0.06) was significantly higher during the growth period from day 48 to day 55 and day 76 to day

Table 1. *Myrothecium* leaf spot severity as influenced by pruning time and shoot age

Pruning time		Leaf spot severity (%)/Shoot age (days)							
Month	Week	48	55	62	69	76	83	90	Mean
March	I	0.000	0.000	0.000	0.000	0.000	0.270	0.590	0.123
	II	0.000	0.000	0.000	0.087	0.363	1.310	3.207	0.710
	III	0.000	0.000	0.000	0.460	1.070	2.833	4.177	1.220
	IV	0.000	0.000	0.347	1.610	2.190	7.467	10.703	3.188
April	I	0.000	0.573	2.253	5.113	8.747	17.350	18.867	7.558
	II	0.000	0.087	4.697	3.417	7.227	13.207	14.723	6.194
	III	0.320	1.740	2.400	9.607	11.00	12.663	14.350	7.440
	IV	0.700	2.737	3.007	3.067	6.480	6.127	6.223	4.049
May	I	1.227	1.480	1.420	4.380	3.467	2.343	3.787	2.587
	II	1.173	3.147	4.603	4.393	2.390	2.840	4.273	3.260
	III	1.347	1.877	2.550	1.507	1.777	4.907	4.953	2.702
	IV	1.077	1.777	1.117	3.380	1.683	4.687	7.540	3.037
June	I	2.417	1.383	2.412	1.533	2.617	4.083	4.487	2.705
	II	1.210	3.917	1.487	2.510	4.407	3.160	5.767	3.208
	III	1.687	2.340	4.23	7.240	4.330	11.520	7.570	5.559
	IV	1.703	3.607	4.093	3.313	5.700	11.340	5.030	4.970
July	I	1.977	3.227	5.197	4.460	5.517	3.800	1.867	3.720
	II	1.253	2.343	2.340	4.437	4.317	5.293	7.200	3.883
	III	2.220	2.113	2.600	3.840	3.463	4.577	6.603	3.631
	IV	1.050	1.553	1.450	2.177	3.433	4.423	7.220	3.044
August	I	1.320	2.343	2.977	2.887	2.957	5.193	5.170	3.264
	II	0.757	2.893	4.450	3.050	2.987	5.540	2.927	3.229
	III	1.637	2.893	4.440	3.490	3.552	2.690	3.213	3.131
	IV	3.397	3.000	3.673	4.283	4.360	2.663	3.750	3.590
Mean		1.103	1.876	2.573	3.343	3.918	5.845	6.425	
CD at 5%									
Date of pruning		0.80							
Shoot age		0.42							
Date of Pruning × Shoot age		2.11							

83. Infection rate was less in day 69 to 76 and day 83 to 90. Mean rate of infection was not varied significantly during the growth period from day 55 to day 76. Average apparent rate was higher (0.091) in plants pruned during first week of April and minimum in plants pruned during first week of July (0.002). Pruning date was however not significantly influenced on the infection rate (Table 2).

Analysis of variance revealed significant effect of growth period ($F = 3.67$, $P < 0.01$) and growth period × date of pruning interaction ($F = 3.20$, $P < 0.01$) on apparent infection rate (r) per unit area per day.

Stepwise multiple regression analysis was performed to find out the sub set of environmental variables that influenced *Myrothecium* leaf spot severity, based on closeness to predict and actual disease severity (Table 3). The R^2 values ranged from 0.58 to 0.60. On the basis of contribution of different weather factors, the linear prediction equation $Y = -81.803 + 1.1768\ x_2 + 0.7656\ x_3$ was selected as the best fit equation for ($R^2 = 0.58$) for predicting the disease under natural epiphytotic condition. Where, x_2 is minimum temperature and x_3 is maximum humidity. A diagrammatic representation of the prediction model made using the predicted values derived from the equation and the actual observed disease severity (Fig. 1).

There was a significant ($P < 0.05$) reduction in number of spots with the increase in distance from the source of inoculum. Maximum number of spots (18 – 25) was found on the trap plants placed 20 cm away from the source and minimum in plants placed 100 cm away from

Table 2. Apparent infection rate of *Myrothecium roridum as* influenced by plant growth period and pruning date

Pruning date		Apparent infection rate per unit area per day						
		Plant growth period (days)						
Month	Week	48-55	55-62	62-69	69-76	76-83	83-90	Mean
March	I	0.000	0.000	0.000	0.000	0.000	0.119	0.020
	II	0.000	0.000	0.000	0.016	0.179	0.141	0.056
	III	0.000	0.000	0.000	0.050	0.140	0.059	0.041
	IV	0.000	0.000	0.105	0.045	0.182	−0.011	0.054
April	I	0.000	0.194	0.145	0.080	0.111	0.018	0.091
	II	0.000	0.156	−0.047	0.114	0.099	0.018	0.056
	III	0.036	0.048	0.211	0.021	0.024	0.018	0.060
	IV	0.198	0.012	0.001	0.114	−0.009	−0.030	0.048
May	I	0.025	−0.010	0.172	−0.035	−0.058	0.071	0.028
	II	0.141	0.060	−0.006	−0.113	0.046	0.062	0.032
	III	0.051	0.044	−0.084	0.027	0.154	0.002	0.033
	IV	0.066	−0.057	0.161	−0.105	0.148	0.076	0.048
June	I	−0.110	0.111	−0.070	0.082	0.066	0.011	0.015
	II	0.174	−0.145	0.079	0.085	−0.054	0.097	0.039
	III	0.036	0.099	0.080	−0.076	0.151	−0.071	0.037
	IV	0.146	0.006	−0.024	0.087	0.107	−0.134	0.032
July	I	0.072	0.056	−0.008	0.033	−0.056	−0.106	−0.002
	II	0.083	−0.004	0.112	−0.004	0.009	0.064	0.044
	III	−0.001	0.022	0.068	−0.014	0.042	0.055	0.028
	IV	0.050	−0.008	0.063	0.068	0.039	0.068	0.047
August	I	0.085	0.017	−0.002	−0.005	0.108	−0.000	0.034
	II	0.203	0.049	−0.042	−0.003	0.095	−0.113	0.032
	III	0.142	0.067	−0.037	0.004	−0.045	0.028	0.027
	IV	−0.034	0.046	0.026	0.002	−0.075	0.051	0.003
Mean		0.057	0.032	0.038	0.020	0.059	0.020	
CD at 5%								
Date of pruning			Not significant					
Shoot age		0.025						
Date of Pruning × Shoot age		0.121						

Table 3. Functional relationship of environmental factors with *Myrothecium* leaf spot severity

Sl. No.	Multiple regression function	R^2
1	$Y = -28.291 + (1.222^*)x_1 + (2.0323^{**})x_2 + (0.4943^*)x_3 + (-0.1649)x_4 + (0.0247)x_5$	0.6062
2	$Y = -30.465 + (-1.3022^*)x_1 + (2.12180^{**})x_2 + (0.5200^{**})x_3 + (-0.1456)x_4$	0.6015
3	$Y = -38.74\ 9 + (-0.8326^*)x_1 + (1.6276^{**})x_2 + (0.4560^*)x_3$	0.5954
4	$Y = 17.09 + (-1.5330^{**})x_1 + (2.016^{**})x_2$	0.5712
5	$Y = -81.803 + (1.1768^{**})\ x_2 + (0.7656^{**})\ x_3$	0.5778

*Significant at $P < 0.05$, **Significant at $P < 0.01$.
Where: x_1 = maximum temperature (°C), x_2 = minimum temperature (°C), x_3 = maximum humidity (%), x_4 = minimum humidity (%), x_5 = rainfall (mm).

the source (Fig. 2). Highly significant ($P < 0.01$) negative correlation with the number of spots on the trap plants and the distance from the inoculum source was observed.

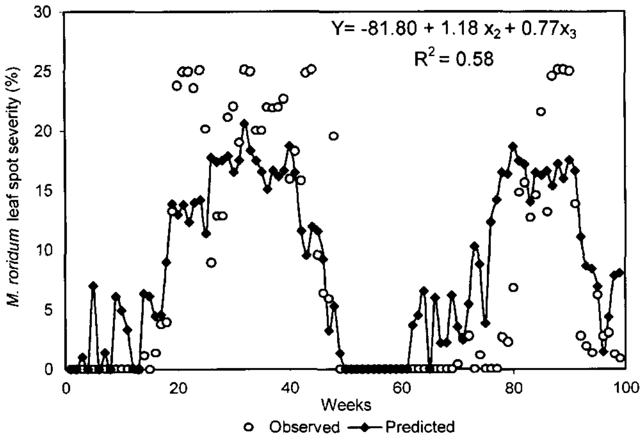

Fig. 1. Prediction model of *Myrothecium roridum* derived from best-fit equation.

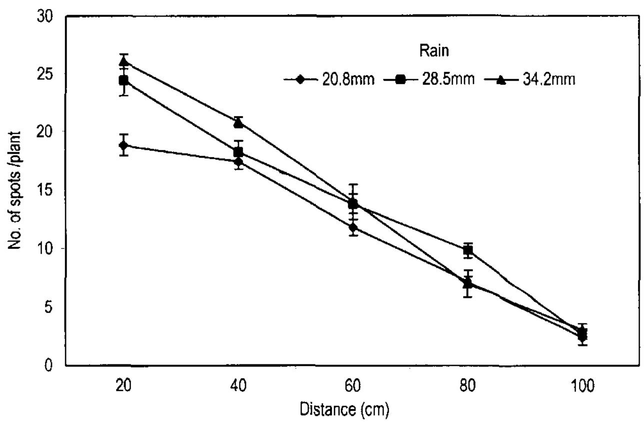

Fig. 2. Dispersal of *Myrothecium roridum* from the source of inoculum.

Discussion

Development of disease depends on the concurrent presence and interaction of a susceptible host, a virulent and aggressive pathogen as well as a favourable environment. Escape from disease depends on the lack of coincidence and interaction of these three factors on a temporary or a permanent basis. Whenever a susceptible plant stage takes place at a different time, *i.e.*, out of phase from the appearance of a virulent pathogen, the plant is likely to escape disease. Escape from disease may also result from early maturing of a variety in relation to the arrival and/ or multiplication of the pathogen (Stakman and Harrar, 1957).

In the present study, severity increased significantly with increase in shoot age. Highest disease severity noticed in 90-day-old shoots and lowest in 48-day-old shoots. Young leaves and fruits are reported to be highly resistant to most facultative pathogens and ageing leaves show a progressive decrease in resistance (Lutterell *et al.*, 1974; Dowley *et al.*, 1975). As leaves age, longer period of leaf wetness is required for infection (Turechek and Stevenson, 1998). Similar result on the higher disease severity in older plants was reported in case of rust infection in mulberry (Sengupta *et al.*, 1990; Pratheesh Kumar *et al.*, 2000).

Escape from the disease may occur as a result of "lateness" in a crop *i.e.*, the susceptible stage in a plant may appear after the pathogen inoculums has been weakened or exhausted (Miller and Bollen, 1946). Maximum severity of *Myrothecium* leaf spot was observed in plants pruned during the first week of April and plants pruned before fourth week of March showed significantly less disease severity. Plants pruned during the first week of April might have gone through a period when more virulent spores of *Myrothecium roridum* are available for infection. Generally, the rain starts in West Bengal during the month of June, which is an important factor for spread of Myrothecium leaf spot, and hence the disease appears with the onset of monsoon.

Plants may escape disease because of too low rain fall or relative humidity, because some disease require a film of water on the plant or high relative humidity in almost every stage of pathogens life cycle. Disease escape often results when the duration of plant wetness is shorter than required by the pathogen (Agrios, 1980). Generally, leaves of mulberry shoots with an age between 60 – 90 days are utilized for silkworm rearing. In this experiment, plants pruned at various weeks passed through different weather conditions, which may be favourable or unfavourable for the pathogen growth. The interaction between the shoot age, and the favourable or unfavourable condition for pathogen growth explains the variation in disease severity observed in plants pruned during different weeks.

The significant ($P < 0.01$) influence of shoot age and shoot age x pruning date interaction observed in this study indicates influence of both environment and host age on the infection rate (r). Infection rate can vary during epidemic of a disease (Gassert, 1976). The rate of infection per unit area per day was noticed higher initially. Many workers made similar observation in various crops (Barbetti, 1981; Hegde and Anahosur, 1994). In this study, higher rate of apparent infection per unit area per day was in young plants rather than in the mature old ones. This may be due to the establishment and colonization of the pathogen in the early growing stage and thereafter growth of pathogens at a steady rate. The fungi utilise food materials available in the leaves for the establishment and colonization. This cause a deficit in food materials required for the subsequent faster growth of the pathogen reducing in the apparent rate of infection in more mature and older plants. Coupled with this, gradual increase in the density of pathogen as reported due to leaf rust disease of mulberry (Pratheesh Kumar *et al.*, 2000) might have caused a slower rate of infection in older plants. Furthermore, Bell (1980) reported that when the pathogen enter various parts of the plants, defense of the leaves against pathogen increase with age.

Environment influence the epidemics of diseases through its various structural elements. Very often, the environmental factors have relative rather than absolute effect on epidemics. This favourable level of one or more factor may compensate for the certain deficiency in other factors (Rotem *et al.*, 1971). The variation in the infection rate on plants pruned at a certain week during its growth period may be due to various favourable or unfavourable environmental factors along with the host physiological factors that existed during the growing season.

Rain has been reported to play major role in the dispersal of fungal spores (Huber *et al.*, 1997; Huber *et al.*, 1998). In this experiment, trap plants placed near the inoculum source received more inoculum. Similar reports on the decrease of number of spores with increase in distance from the source were reported in case of rust fungi from infected wheat plants. (Geagea *et al.*, 1999). Further spore deposition gradients of rain-splashed spores are usually steeper than those of wind-dispersed spores where few rain-splashed spores go beyond 1 meter (Gregory, 1973; Madden, 1992).

Since this disease affect on silkworm rearing during the rainy season, early utilization of leaves and change of pruning schedule to minimize the disease severity can be practiced for getting good quality mulberry leaves for silkworm rearing.

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