Proc. Indian natn. Sci. Acad. **B52** No. 5 pp 689–694 (1986)

Comparative Study of Phylloplane Mycoflora of *Cercospora beticola* Infected and Non-infected Leaves of *Spinacia oleracea* and Factors affecting Disease Severity

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(Received 4 April 1986; after revision 5 October 1986)

Leaves of *Spinacia oleracea* infected by *Cercospora beticola* harboured more fungal population/cm^2 leaf surface and more fungal species than non-infected leaves. There was a characteristic mycoflora on the leaf surface of *Spinacea oleracea*. Some fungi were restricted either to infected or non-infected leaves. Disease severity was greatly influenced by atmospheric temperature, humidity and age of the plant.

Key Words : Phylloplane, Mycoflora, Infected, Non-infected, *Spinacia oleracea*

Introduction

Interactions between microorganisms on the leaf surfaces usually have been considered in relation to the influence of saprophytes on the parasites (Fokkema 1978, Mukerji 1984). There are a few reports available regarding the influence of the leaf pathogens on the saprophytic microorganisms (Mckenzie & Hudson 1976, Singh et al. 1984). Leaf spot of *Spinacia oleracea* incited by *Cercospora beticola* is a serious foliar disease in northern India. Therefore, an attempt has been made to evaluate the possible differences between the phylloplane mycoflora of *Cercospora beticola*-infected and non-infected leaves of *Spinacia oleracea* L. and histopathological and physiological factors affecting the *Cercospora* leaf spot disease.

Materials and Methods

Seeds of spinach (*Spinacia oleracea* L. CV All green) were sterilized by 0.1% mercuric chloride, washed thoroughly in sterilized distilled water and sown in earthen pots (18 × 25 cm). Only five plants per pot were maintained. Ten pots were used for each treatment. The infected plants were obtained by artificially inoculating them with *Cercospora beticola*. To obtain an adequate supply of conidia, *C. beticola* infected leaves were detached from the plants, lesions were cut by sterilized scissors, surface sterilized and placed in Petri dish moist chambers for 48 hr at 25 ± 1°C. These lesions were washed in sterilized distilled water and strained through muslin cloth to get conidial suspension. From this, further dilutions were made in sterilized distilled water (approximate ratio of 3000 conidia/ml).

At the age of 45 days, the plants grown in earthen pots were inoculated by spraying the inoculum on the foliage until leaves were thoroughly wetted. The pots with inoculated plants were covered with polythene bags for 48–72 hr to get good lesions. The control plants were sprayed with sterilized distilled water and also covered with polythene bags. Finally, infected and non-infected plants were kept in green house.

First collection of *C. beticola* infected leaves was made when spots appeared 15 days after infection. The second collection was made when lesions were mature and third sampling when infected tissues senesced and died. For isolation of phylloplane mycoflora four methods, viz. dilution plate (Singh & Rai 1980), serially washed leaf discs (Harley & Waid 1955), surface-sterilized leaf discs (Pugh & Buckley 1971), and moist chamber techniques (Keyworth 1951) were used.

Results and Discussion

The non-infected and infected leaf surfaces represented different microhabitats. A perusal of the mycoflora isolated separately from *Cercospora beticola* infected and non-infected leaves of *Spinacia oleracea* sampled simultaneously, showed that infected leaves harboured larger fungal population/cm^2 leaf surface and more fungal species than non-infected leaves (figure 1).

Sadasivam and Prasad (1973) compared the phyllosphere population of diseased (infected by *Cercospora henningsii)* and healthy leaves and noticed that fungal population increased manifold on the diseased leaves compared to the healthy ones of tapioca. Srivastava and Bisht (1982) also recorded higher fungal counts on powdery mildew-infected leaves of pea and fenugreek than on non-infected leaves. Our results are in agreement with those of Hayes (1982), Singh et al. (1984) and Parker and Blakeman (1984). Increase in the fungi on infected leaves can be attributed to the fact that after infection the nature of leaf exudate could have undergone changes due to altered metabolism of infected leaves. It was also observed that infected leaves senesced relatively earlier than the non-infected ones.

The phylloplane mycoflora of non-infected, and *Cercospora*-infected leaves showed somewhat distinct groups of fungi. While some fungi were common to both infected or non-infected leaves, by more number of colonies/cm^2 and % frequency of occurrence, others were restricted either to infected or non-infected leaves. Some fungi even showed more or less equal number of colonies/cm^2 and % frequency of occurrence, as also reported by earlier workers (Pal 1981, Srivastava & Bisht 1982, Singh et al. 1984). This change in mycoflora may be due to the fact that *Cercospora* infection induces changes in chemical composition of the host which are advantageous to some saprophytes and disadvantageous to others.

Most of the fungi present in smaller number on healthy leaves were represented by a very large number on the infected ones. For example, *Alternaria alternata, Aspergillus niger, Cladosporium cladosporioides, Fusarium oxysporum, Trichoderma lignorum* which were present in smaller number on healthy, non-infected leaves were represented by higher number and percentage frequency on infected leaves (figure 2). On *Cercospora* infected leaves of tapioca, *Alternaria* sp., *Curvularia* sp., *Helminthosporium* sp. and mycelia sterilia were more common than on non-infected leaves (Sadasivam & Prasad 1973). Mckenzie and Hudson (1976) isolated *Alternaria* sp., *Gonatobotrys simplex, Verticillium* sp., *Ramularia* sp. in greater number from rust-infected material of poplar and plum leaves and wheat stems. Wadje and Deshpande (1979) observed higher incidence of *Alternaria tenuis, Cladosporium herbarum* and *Phoma exigua* on *Xanthomonas*-infected leaves than on non-infected leaves of cotton. On the other hand, *Aspergillus flavus, Candida albicans* and *Epicoccum purpurascens* occurred in larger number of colonies/cm^2 leaf surface and higher percentage frequency on non-infected leaves than infected leaves. *Melanospora* sp. was restricted only to non-infected leaves.

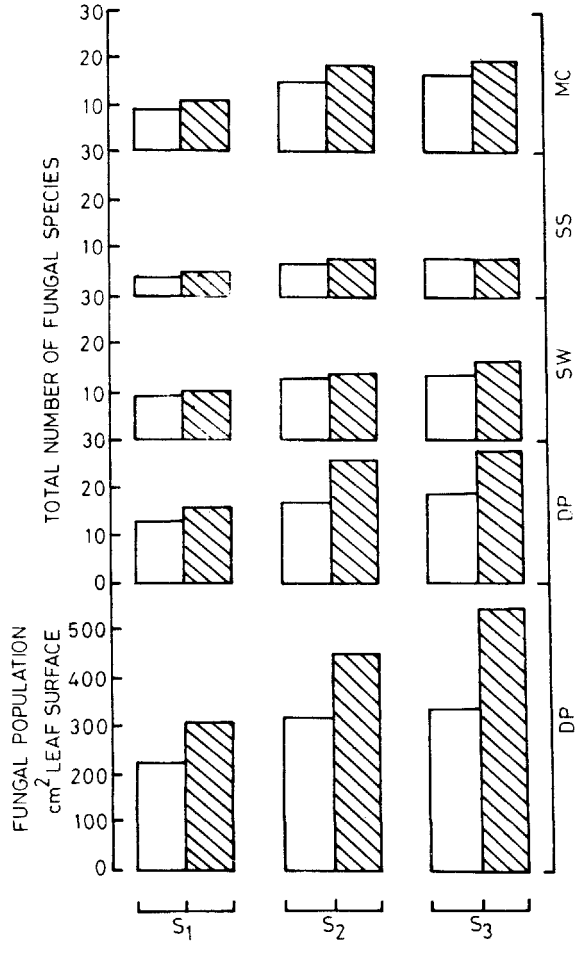

Figure 1 Total fungal population/cm^2 leaf surface and total number of fungal species on non-infected and *Cercospora*-infected leaves of *Spinacia oleracea*. (□), Non-infected leaves; (▨), *Cercospora*-infected leaves **DP,** Dilution plate method; **SW,** Serially washed leaf discs method; **SS,** Surface-sterilized leaf discs method; **MC,** Moist chamber method

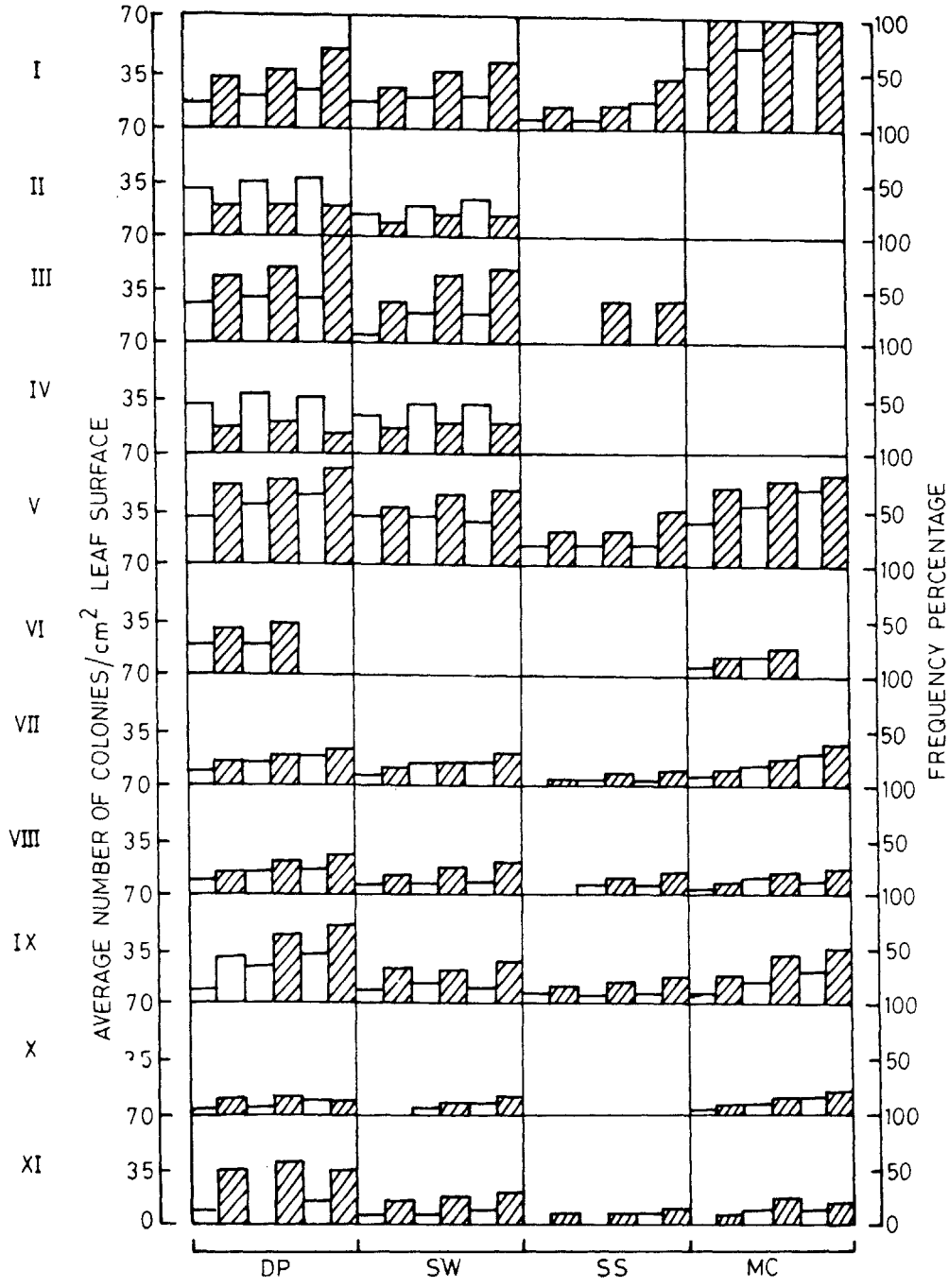

Figure 2 Occurrence of some phylloplane fungi on non-infected and *Cercospora*-infected leaves of *Spinacia oleracea*. (□), Non-infected leaves; (▨), *Cercospora*-infected leaves;
I, *Alternaria alternata;* II, *Aspergillus flavus;* III, *A. niger;* IV, *Candida albicans;* V, *Cladosporium cladosporioides;* VI, *C. herbarum;* VII, *Curvularia lunata;* VIII, *Drechslera australiensis;* IX, *Fusarium oxysporum;* X, *Nigrospora sphaerica;* XI, *Trichoderma lignorum*
DP, Dilution Plate method; **SW,** Serially washed leaf discs method; **SS,** Surface-sterilized leaf discs method; **MC,** Moist chamber method

Table 1 *Presence (+) or absence (−) of fungi isolated from non-infected (N) and* Cercospora*-infected (C) leaves of* Spinacia oleracea *isolated by the dilution plate (DP), serially washed leaf discs (SW), surface-sterilized leaf discs (SS) and moist chamber (MC) methods*

Name of the fungus	Methods							
	DP		SW		SS		MC	
	N	C	N	C	N	C	N	C
Alternaria alternata (Fr.) Keissler	+	+	+	+	+	+	+	+
Aspergillus flavus Link ex Fries	+	+	+	+	−	−	−	−
A. fumigatus Fresenius	−	+	−	−	−	−	−	−
A. luchuensis Inui	−	+	−	−	−	−	−	−
A. nidulans (Eidam) Winter	+	+	−	−	−	−	−	−
A. niger Van Tieghem	+	+	+	+	−	+	−	−
A. terreus Thom.	−	+	−	−	−	−	−	−
Candida albicans Berkhout	+	+	+	+	−	−	−	−
Cephalosporium curtipes Sacc.	−	−	−	−	−	−	+	+
Cercospora beticola Sacc.	−	−	−	−	−	−	−	+
Chaetomium globosum Kunze ex Fries	+	+	+	+	−	+	+	+
Cladosporium cladosporioides (Fresen.) de Vries	+	+	+	+	+	+	+	+
C. herbarum (Pers.) Link ex Gray	+	+	−	−	−	−	+	+
Colletotrichum sp.	−	−	−	−	−	−	+	+
Curvularia lunata (Wakker) Boedijn	+	+	+	+	+	+	+	+
C. pallescens Boedijn	−	+	−	−	−	−	−	+
Drechslera australiensis (Bugnicourt) Subram. and Jain ex Ellis	+	+	+	+	+	+	+	+
D. hawaiiensis (Bugnicourt) Subram. and Jain	−	+	−	−	−	−	−	−
Doratomyces sp.	−	+	−	−	−	−	−	−
Epicoccum purpurascens Ehrenb. ex Iclecht	+	+	−	−	−	−	+	+
Fusarium oxysporum Schlechtendahl	+	+	+	+	+	+	+	+
Graphium sp.	−	+	−	−	−	−	−	−
Humicola brevis (Gilman and Abbott) Gilman	+	+	−	−	−	−	−	−
Melanospora sp.	+	−	−	−	−	−	+	−
Memnoniella echinata (Riv.) Galloway	−	−	−	−	−	−	−	+
Mucor racemosus Fresenius	+	+	+	+	−	−	−	−
Myrothecium indicum Rama Rao	+	+	+	+	−	−	+	+
Nigrospora sphaerica (Sacc.) Mason	+	+	+	+	+	+	+	+
Penicillium sp.	+	+	−	+	−	−	−	−
Pestalotia sp.	+	+	−	−	−	−	+	+
Phoma humicola Gilman & Abbott.	+	+	+	+	+	+	+	+
Rhizopus arrhizus Fischer	+	+	+	+	−	−	−	−
Sclerotium sp.	−	+	−	−	−	−	−	−
Stachybotrys atra Corda	−	−	−	−	−	−	+	+
Staphylotrichum sp.	−	+	−	−	−	−	−	−
Sterile hyphae (Brown)	−	+	−	+	−	−	−	−
Thielaviopsis sp.	−	+	−	−	−	−	−	−
Torula herbarum (Pers.) Link ex Gray	+	+	−	−	−	−	+	+
Trichoderma lignorum Oudemans	+	+	+	+	+	+	+	+
Trichothecium roseum Link.	−	−	−	−	−	−	+	+

Few species (*Aspergillus fumigatus, A. luchuensis, A. terreus, Cercospora beticola, Curvularia pallescens, Doratomyces* sp., *Drechslera hawaiiensis, Graphium* sp., *Memnoniella echinata, Sclerotium* sp., *Thielaviopsis* sp. and brown sterile hyphae were confined to *Cercospora*-infected leaves only (table 1).

The difference in fungal population observed in the present study seems to depend mainly upon two things: (i) competition associated with antagonism with other saprophytic forms colonizing leaves at the same time and (ii) suitability of the substrate at a particular stage of leaf decomposition due to infection (Mukerji 1974). For example, we observed that after the infection, the leaf spots varied from few to many per leaf; they were small, circular to oval, had a definite margin, and big patches measuring 2–5 mm in diameter. Leaf tissues adjacent to the margin frequently turned reddish brown. After 20–30 days of infection, closely situated spots coalesced and formed large circular or irregular necrotic patches. Later, they became papery, thin and brittle in the centre and necrotic cells were collapsed. When the spots were numerous, the whole leaf senesced, died prematurely and dropped.

The degenerative process within the leaf began with the entrance of pathogen. The penetration through stomata took place without the formation of appressoria. There was no evidence of direct penetration. Solel and Minz (1971) reported that appressoria were formed over stomata prior to penetration. Feindt et al. (1981) observed that penetration took place through stomata. We observed that after stomatal penetration germ tubes continued to grow by branching repeatedly and ramify within the tissue. It could not be ascertained whether penetrating hyphae became intracellular immediately or they were at first intercellular. Invaded cells died quickly forming necrotic lesions. Cell wall showed varying levels of suberization. Cunningham (1928) also noted cell wall thickening which he attributed to lignification and suberization. The cell collapse increased progressively with the degenerative sequence. The fungal hyphae often extended from the necrotic central area of the lesion into the boundary region. The conidiophores emerged through the stromata. More often, fascicles of 3–12 or sometimes more conidiophores emerged from a dense cluster of round to irregular brown cells. The filamentous hyphae were ramified throughout the lesion usually in the intercellular spaces. Latham (1934) in his study of *Cercospora* sp. leaf spot of cowpea reported that only after cells reached a holocentric condition, did the fungus become intercellular. Sindhu (1985) observed that disease severity of *Cercospora* was influenced greatly by atmospheric temperature, humidity and plant age. High disease severity was observed in the month of October-November when maximum and mimimum temperatures were in the range of 33–29°C and 14–12°C respectively. With increase in the period of high humidity (72 and 96 hr) and plant age, *Cercospora* infection was also increased. This changed environmental condition might have influenced more fungi in the phylloplane of infected leaves.

The cell wall of necrotic cells appeared to have been partially dissolved. Infrequently, cells with broken cell walls in the intercellular spaces were observed. It was not clear whether these changes were a direct effect of the fungus or were associated with host cell autolysis. But progressive degenerative process of the host tissue may change ecological substrate resulting in distinct mycoflora in the infected region.

Acknowledgements

The authors are grateful to Professor K G Mukerji, Head of the Botany Department, University of Delhi, for valuable suggestions. Thanks are also due to the Head of the Botany Department, Meerut College, Meerut for providing laboratory facilities. One of them (IRS) is thankful to the Council of Scientific & Industrial Research.

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