Microflora of Leaf Surface in Relation to Virus Infection

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The study of leaf surface microflora has attracted the attention of different workers in recent years. The leaf surface being nutritionally very rich offer a suitable substratum for the colonization of the various microbes both parasites and saprophytes. Studies relating to mutual antagonism of the micro-organisms and the possibilities of biological control of some pathogens opened a new field of investigation to the pathologists. Earlier workers (Last, 1955; Dickinson, 1965, 1967 and Ruinen, 1961, 1965, 1967) reported the microflora of different plant species without laying much emphasis on the mutual interaction of the micro-organisms associated with leaf surface. Recently Mishra and Srivastava (1968, a, b, c, d) reported the phyllosphere and phylloplane microflora of some important crop plants and observed that bacterial population mostly have suppressing effect on fungal flora of leaf. The interaction of the microflora and the virus (viruses) associated with leaf surface has not been studied earlier and this forms the basis of the present investigation.

Materials and Methods

Two plant species, i. e. *Croton bonplandianum* and *Petunia hybrida* infected by an unidentified virus (viruses?) and CMV. respectively were selected for the present investigation. The former species was collected directly from the field inside the University Campus, where it was growing luxuriently and the latter was obtained from pots where it was raised from the seeds and inoculated by the virus. Infected leaves were sampled at random, brought to the laboratory and phyllosphere and phylloplane microflora were investigated by the methods described earlier (Mishra and Srivastava, 1968, b). Simultaneously leaves from the healthy plants growing near to diseased ones were also sampled and inoculated for comparison. Fungal and bacterial population was assessed using modified Martin's and Czapek's (pH 8—8.2) media respectively. The plates were incubated 6 and 2 days for fungi and bacteria respectively.

Results

From Table 1 it is evident that the number of fungal species associated with diseased and healthy leaves of *Croton* was highest in the beginning and least in the middle period of sampling. The bacterial population on the other hand exhibited different pattern in the two types of leaves. It was highest in the beginning on the diseased leaf and decreased continuously but the population was least in the beginning in case of healthy leaf and highest in the middle.

The pattern of distribution of the microflora on the *Petunia* leaves was of some what different type. In diseased leaves the number was maximum in the beginning and decreased continuously whereas in healthy leaves it was least in the beginning and the maximum in the middle. Bacterial population was maximum in the beginning in both the types of the leaves. In diseased leaves it dropped off continuously but in case of healthy the population again increased in the end (Table 3).

The fungal species associated with two types of *Croton* phyllosphere varied. The variation in dominants was also observed at different sampling periods (Table 1). In *Petunia* for most of the time *Cladosporium* spp. dominated the phyllosphere region and only once *Penicillium* spp. and *Fusarium* spp. occurred with higher percentage (Table 3).

Phylloplane region of *Croton bonplandianum* exhibited variation in distribution pattern and type of the fungal species particularly the dominant ones. The number of fungal species was generally higher in the beginning and decreased lateron. The dominant species, however, varied at different stages. Different dominant species were associated with the phylloplane region of diseased and healthy leaves at the different sampling periods (Table 2).

Discussion

The variation in the type of the dominants associated with the two types of leaves at different stages is possibly due to differences in their physiological conditions. Leaf exudation primarily responsible for the microbial population possibly varied in the two types of leaves (i. e. healthy and diseased). Besides this, the leaf tissue harbouring the microbes also varied in healthy and diseased leaves. The tissue system in the latter type of leaves gets disturbed by the virus infection and there by a possible variation in the microflora might be well expected. The bacterial population is generally adversely affected by the severity of the virus infection and the former has depressing effect upon fungal flora. A comptex interaction between the various micro-organisms i. e. fungi, bacteria and virus (viruses) was observed. The role of bacteria and fungal population upon virus infection and their spread is under investigation and will be published lateron.

Summary

Phyllosphere and phylloplane microflora of virus infected plants of *Croton bonplandianum* and *Petunia hybrida* has been studied. In diseased leaves of both plants, the number of fungal isolates was higher in beginning and decreased lateron when the severity of disease increased. In case of healthy leaves of *Croton* the number of fungal species was maximum in the beginning and least in the middle while in *Petunia* the trend was just reverse. Bacterial population exhibited a depressing effect upon fungal population. A complex interactieon between virus, fungi and bacteria was observed.

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Table 1. Percentage occurrence of fungi in the Phyllosphere of healthy and diseased plants of *Croton bonplandianum*

Isolated fungi	15th Aug. '68 V	H	1st Sept. '68 V	H	10th Oct. '68 V	H	15th Noc. '68 V	H
Rhizopus nigricans	1			15	100	6	1	
Mucor hiemalis		6						
Cunninghemella berthollletiae	1	3						
Chaetomium herbasum	1	5						
Neocosmospora vasinfecta	3							
Phoma hibernica							1	
Aspergillus flavus	14	4		30		6		1
A. sydowi	2		50			26		
A. niger	20	8		45		2	1	
A. aculeatus	4					3		
A. versicolor	3							
A. sulphureus								1
A. flavipes	2	7						
A. phoenicis	1	8						
Penicillium humicola		4						1
Paecilomyces fusisporus		8						
P. varioti		2						
Curvularia lunata	40	5				1	2	10
Alternaria tenuis		4						
Cladosporium spp.						44	94	84
Fusarium spp.	4	4				12	1	1
Black sterile colony	2	32						
Yellow sterile colony	1							
White sterile colony	1		50	10				2
Number of species	16	14	2	4	1	8	6	7

V = Virus infected
H = Healthy

Table 2. Percentage occurrence of fungi in the phyllosphere of healthy and diseased of *Croton bonplandianum*

Isolated fungi	15th Aug. '68 V	H	1st Sept. '68 V	H	10th Oct.'68 V	H	15th Noc. '68 V	H
Aspergillus flavus	10		10	9	40	40	6	
A. sydowi			5	13				
A. niger		12	6		10			
A. ochraceous			42					
A. aculeatus	10							
Monilia geophila			20					
Trichoderma glaucum				7				

Isolated fungi	15th Aug. '68 V	H	1st Sept. '68 V	H	10th Oct.'68 V	H	15th Noc. '68 V	H
Penicillium spiculisporum		2						12
Curvularia lunata	10	8	13	15		10	30	8
Cladosporium spp.							20	66
Alternaria spp.							44	14
Fusarium spp.	50		3					
Black sterile colony	10	2		48				
White sterile colony		70						
Number of species	6	6	7	6	3	3	4	4

Table 3. Percentage distribution of fungi in the Phyllosphere of healthy and virus infected plants of *Petunia hybrida*

Isolated fungi	10th Nov. '67 V	H	10th Dec. '67 V	H	15th Jan. '68 V	H
Rhizopus nigricans	15	5				
Mucor luteus	2		6			
Phoma hibernica			3	4		
Aspergillus niger	4					
A. flavus	16			6	1	10
A. candidus	1				1	
A. sydowi	1			10		
A. tamarii	1			2		
Penicillium spiculisporum	20	10				
Trichoderma glaucum					1	
Cephalosporium sp.				12		
Cladosporium spp.	30	85	70	42	93	34
Alternaria spp.	5		6	12	2	6
Fusarium spp.	3		5	4		40
White sterile colony	2		4	2		4
Number of species	12	3	7	10	6	6

Table 4. Microfloral quantity in the Phyllosphere of different plants

	Croton bonplandianum							
	15 Aug. '68		1 Sept. '68		10 Oct. '68		15 Nov. '68	
	V	H	V	H	V	H	V	H
Bacterial colonies per plate	178	294	130	550	43	314	584	442
Fungal colonies per plate	33	97	2	3	34	4	191	55
Number of fungal species	16	14	2	4	1	8	6	7

	Petunia hybrida					
	10 Nov. '67		10 Dec. '67		15th Jan. '68	
	V	H	V	H	V	H
Bacterial colonies per plate	142	617	94	219	45	491
Fungal colonies per plate	8	4	20	3	64	6
Number of fungal species	13	3	7	10	6	6

V = virus infected
H = healthy